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Preface

GEORGE ROSEN

A DECADE has now elapsed since the antibiotics first began to figure prominently on the medical scene. Characteristic of the period have been the extraordinary expansion of knowledge concerning antibiotics and the breath-taking rapidity with which this knowledge has been applied. The prediction may be ventured that to future medical historians the decade 1941–1951 will appear as the golden period of antibiotic research, much as we today regard the 'eighties and 'nineties of the last century as the golden era of bacteriology.

It seems fitting, therefore, to look back at the decade that has passed, to see what has been accomplished, and how it all happened. In effect, our aim is two-fold: first to present an important phase of contemporary medical history, and secondly to have this story told, if possible, by those who made it. To be sure, this approach has both advantages and weaknesses. The considerable advantage enjoyed by the writer of contemporary history was indicated in sharply exaggerated form by Charles Beard in 1914 when he commented: ". . . the doctrine that we can know more about Andrew Jackson whom we have not seen than about Theodore Roosevelt whom we have seen is a pernicious psychological error." At the same time, the student of contemporary history labors under the disadvantage that he may be too close to his subject to see it in proper perspective. No matter how numerous the facts available, this lack of perspective may result in historical distortion.

Bearing these points in mind we have, nonetheless, undertaken to present the story of a decade of antibiotics. On the basis of the available evidence, we endeavor in this symposium to develop the meaning of the past as a forerunner to and a part of contemporary achievement.

The observations of Alexander Fleming on the penicillium mold in 1929, and the successful extraction of penicillin by Howard Florey and the Oxford group are landmarks in the history of medicine. At the same time, it must be remembered that this work is but one phase of a long historical process which still continues. Implicit in the discovery of antibiotics, which we rightly hail as a great achievement, is the accumulation of some three centuries of scientific knowledge, as well as the corresponding growth of general ideas and social circumstances that have led to this therapeutic goal.

The consequences of this development are clear. The symposium before us testifies eloquently to the large body of specialized knowledge that

now can be marshaled to conquer disease. The next important step is the rapid and effective application of this knowledge to the problems of human biology, and it seems plausible to suggest that this will mark the decade of the 'fifties. The historian of the next decade will have that as his task. Sufficient unto this day is a summary of that which has been accomplished and a glimpse at the problems yet to be solved.

Introduction*

JOHN F. FULTON

THE abrupt expansion of our geographical horizons has precipitated an acute need for an equal expansion of historical perspectives. Not many years ago history, as taught in schools and colleges, consisted largely of a record of rulers, courts, and military expeditions. In English schools, for example, the only history that was taught up to the nineteenth century was that of Greece and Rome, with particular reference to the expeditions of Julius Caesar. In the eighteenth century, Joseph Priestley tried to change all this when he inaugurated, between 1760 and 1768, a course in English history at the dissenting academy at Warrington, near Manchester. He also published several volumes for the instruction of young Englishmen destined to occupy government posts in various parts of the British Empire.[1] He felt that men who were to have such assignments should be thoroughly familiar, not only with their own military history and their kings and queens, but with the social and political history of Britain and of all countries impinging upon the Empire.

Joseph Priestley was ahead of his time in other respects, for not only did he begin to teach social and political history, as we now understand it, but he sought to correlate general history with scientific history, and to this end he published two remarkable historical monographs: the first, a history of electricity[2] (which he undertook on a casual suggestion from Benjamin Franklin, made during one of Franklin's visits to London), and the other a history of optics[3] which was issued several years later. No writer since Priestley's time has been more successful than he in correlating the sequence of scientific development with the chronology of general history—social, political, and military.

And so now, when we find ourselves confronted with a more widespread interest in our historical backgrounds, scientific and otherwise, than at any other time in the history of the world, it behooves us to take cognizance of our responsibility as historians. In the field of science this re-

* Remarks before American Association for the Advancement of Science, St. Louis, 30 March 1946.

[1] Priestley, Joseph. *Lectures on history and general policy; to which is prefixed an essay on a course of liberal education for civil and active life.* Birmingham, 1788. See also: *A description of a new chart of history, containing a view of the principal revolutions of the empire, that have taken place in the world,* which has passed through several editions.

[2] Priestley, Joseph. *The history and present state of electricity with original experiments.* London, 1767.

[3] Priestley, Joseph. *The history and present state of discoveries relating to vision, light and colours.* London, 1772.

sponsibility is particularly great since the public has become more aware than ever before of the scientific forces that move in the world. An increasing number of people are interested in the backgrounds of radio-activity, electronics, and atomic fission; in the medical field they have asked for authentic treatises on the history of malaria and quinine and of other specific diseases and the therapeutic measures essential to combat them. Venereal disease control is no longer barred from public discussion; and it is a rare experience to ride on a New York subway without hearing of a new disease that penicillin or one of the other antibiotics will cure.

The need for general historians is also great since our success in dealing with other nations (and we must inevitably deal with them in view of our present world position) will depend in large measure upon our knowledge of their history and traditions. It is probable that much of our continued diplomatic tangle with the Soviet Union stems from an imperfect knowledge of the history of Russia and the psychological outlook of her people. And the reverse is no doubt also true, for no great power has ever been more gravely handicapped than is Russia at the present moment by ignorance on the part of her leaders of the historical backgrounds and habits of thinking of her democratic allies in the United Nations. Had Hitler been as keen a student of history as Winston Churchill or Franklin D. Roosevelt, World War II might have gone quite differently for the Nazis—or perhaps there would have been no war.

These general considerations have a high degree of relevance to the subject of this symposium which is, in the truest sense of the word, international in its implications, for the original production of penicillin as well as of the other antibiotics represents one of the finest examples of international co-operation to be found in the annals of scientific history. In its own way the development of penicillin has been quite as remarkable as the research which led to the atomic bomb. As Dr. Brunel points out, the beginning of our knowledge of microbial antagonisms goes back to the beginning of bacteriology itself, for it was early recognized that one organism might impair or even arrest the growth of another micro-organism developing in its close vicinity. The term most generally used to designate the antagonisms between micro-organisms is "antibiosis," which was introduced in 1889 by the French bacteriologist Vuillemin.[4] The word has been in sporadic use ever since and Waksman[5] has employed the term "antibiotic" to designate the chemical substance in a given micro-organism re-

[4] Vuillemin, P. Antibiose et symbiose. *C. R. Assoc. franc. Avanc. Sci.*, 1889, pt. 2, 525–543.

[5] Waksman, S. A. *Microbial antagonisms and antibiotic substances.* New York, Commonwealth Fund, 1945.

sponsible for arresting growth in the other organism. Specifically, then, any chemical substance which inhibits growth is an antibiotic, and the sulpha drugs, or for that matter any chemical antiseptic, may be so regarded.

When the late William Welch was called upon to give the first Ether Day address in Boston,[6] he was obliged to discuss the uncomfortable topic —and it has always been a particularly uncomfortable theme in Boston— of who discovered surgical anesthesia. Welch was thoroughly versed in the history of the subject. He knew of Humphry Davy's work on nitrous oxide, of Crawford W. Long's use of ether as a surgical anesthetic in 1844, of Horace Wells' attempts to employ nitrous oxide for tooth extractions in December, 1844, and of the rival claims of Charles Jackson, the Harvard chemist, who had supplied Morton with his ether. Welch took the realistic position that the credit for major discoveries must be given, not to the man who first has the idea, nor to the one who, having had the idea, fails satisfactorily to prove it, but rather to the investigator who, in assuming public responsibility for the demonstration of his discovery, first convinces the world. In Welch's opinion the man who first proved to a skeptical public that surgical anesthesia was both safe and feasible was the Boston dentist, William T. G. Morton. Following the same line of reasoning we are driven inescapably to the conclusion that credit for the *introduction of penicillin as a therapeutic agent* goes not to Pasteur, nor Gratia, nor Fleming, nor to the many others about whom you will read, but rather to those who first isolated penicillin and gave clear-cut proof of its clinical usefulness, its assay and dosage, as well as the mode of its excretion from the body.[7]

Accidental contamination of a pure bacterial culture by another microorganism has been a frequent occurrence since the beginning of bacteriological study. In 1928, for example, Professor Alexander Fleming of St. Mary's Hospital in London accidentally allowed several of his petri dishes on which pathogenic organisms were growing to remain uncovered before an open window and the next day it was found that they had been contaminated by an airborne fungus, later identified as the mold *penicillium notatum*.[8] He observed that wherever the mold had begun to grow, the bacterial colonies near-by had either shrunk or disappeared. Instead of casting the contaminated cultures aside, he made note of the growth inhibition, finding that it extended for some distance beyond the actual growth

[6] Welch, W. H. *A consideration of the introduction of surgical anesthesia.* Boston, 1908.

[7] Florey, H. *et al. Antibiotics.* London, Oxford University Press, 1949. 2 vols.

[8] Fleming, A. On the antibacterial action of cultures of a Penicillium, with a special reference to their use in the isolation of B. influenzæ. *Brit. J. exp. Path.*, 1929, *10*, 226–236.

of the mold. From this he concluded that the mold must give forth a chemical substance which diffuses for some distance into the culture medium. Realizing that there might be therapeutic possibilities if the agent which had inhibited the bacterial growth could be extracted from the mold, Fleming set about to grow the mold in broth and to make extracts of the broth in the hope of capturing the active growth-inhibiting principle. In the course of his studies he found that whatever was elaborated by the *penicillium* mold served to inhibit most of the gram-positive pathogenic organisms which included streptococcus, staphylococcus, meningococcus, and the gram-negative gonococcus. He also observed that the soil organisms, interestingly enough, were resistant to the growth-inhibiting agent of the *penicillium* mold and he was quick to realize that this would be highly useful for differential culture, since growth of soil organisms is difficult owing to frequent contamination by the airborne pathogens. Hence, by merely adding the crude broth in which *penicillium* had grown to his culture medium, the growth of pathogens was inhibited and the soil organisms were allowed free play.

Fleming, believing that the growth-inhibiting effects were due to a specific agent in the mold, named the agent "penicillin." He and his associates, Clutterbuck, Lovell, and Raistrick,[9] attempted to extract the penicillin, but their efforts were largely unsuccessful and by 1932 they were forced reluctantly to conclude that penicillin was too "labile" for extraction or for therapeutic use. In addition to ascertaining what classes of organism would be affected by his penicillin broth, Fleming found that it was destroyed by heat and he also established, through animal experimentation, that penicillin must be relatively non-toxic to animals since the crude broth could be injected intravenously without obvious toxic effect. Here the subject was left, and apart from the occasional use of penicillin broth for differential culture of soil organisms, Fleming made no further attempt to extract penicillin or to establish its therapeutic usefulness.

The story of the introduction of penicillin for therapeutic use has three primary phases. The first lies in the initial observations of Fleming, just outlined, which culminated in the naming of an agent not otherwise identified, but in failure to apply it therapeutically. Fleming's work, however, served to refocus world attention upon an old problem. I find, for example, among the records of the Yale School of Medicine an application made in 1930 by

[9] Clutterbuck, P. W., Lovell, R., and Raistrick, H. Studies on the biochemistry of micro-organisms; the formation from glucose by members of the Penicillium chrysogenum series of a pigment, an alkali-soluble protein and penicillin—the antibacterial substance of Fleming. *Biochem. J.*, 1932, *26*, 1907–1918.

a man who has since become a prominent member of our faculty for a grant to study the possibility of extracting the *penicillium* mold for therapeutic purposes. The grant was turned down.

The second phase in the penicillin story lies in the victory of Howard Florey and his resourceful team, who showed how to obtain penicillin in relatively pure form and who demonstrated its clinical usefulness. The third and equally important development was the large-scale production of the drug which, thanks to the vision of A. Newton Richards, was brought about in a surprisingly brief space of time.

Sir Howard came to this country requesting help in the commercial production of penicillin. He came with the backing of the Rockefeller Foundation which in 1938 had recommended a grant of $1200 to support his projected study on the antibiotic. Florey and his associate, Norman Heatley, arrived on 3 July 1941 and were immediately put in touch with the chairman of the National Research Council, Dr. Ross G. Harrison, Professor Emeritus of Zoology at Yale. Harrison, although not clinically trained, saw at once the broad biological implications of Florey's work, since it involved basic rivalry between two large groups of living things, namely, the plant molds and the bacteria. He sent Florey and Heatley to the Department of Agriculture which in turn placed at their disposal the well-equipped government mold laboratories at Peoria, Illinois. There for three months Dr. Heatley and Robert D. Coghill, Director of the Fermentation Division of the Laboratory, explored methods for extracting penicillin. By introducing a new step in the original Oxford procedure they were able to increase the yield of penicillin tenfold.

By the end of September, 1941, Florey was satisfied that everything was proceeding smoothly and he turned over the supervision of the therapeutic testing of penicillin to Dr. Lewis H. Weed, Chairman of the Division of Medical Sciences of the National Research Council, and through him to the Council's Committee on Chemotherapeutic and Other Agents. Dr. Perrin Long was originally Chairman of this committee; in May, 1942, Dr. Long entered the Army and was succeeded by Dr. Chester Keefer of Boston, who became responsible for directing the broadly conceived program of therapeutic testing of penicillin and other chemotherapeutic substances.

Those were days of great scarcity of penicillin. It was in constant demand by the armed forces, and Dr. Keefer had the unenviable task of rationing the meager supply available in 1942–1943—even to refusing frantic requests to save the lives of his friends and his friends' children. The early distribution was handled wisely and fairly, and there was no justifiable

criticism of the way in which this heavy responsibility was executed.

The production program was sponsored energetically by Dr. A. N. Richards as Chairman of the Committee on Medical Research of the Office of Scientific Research and Development (OSRD). The OSRD had come into being only three days before Dr. Florey's arrival in this country, but the Committee on Medical Research did not become fully organized until about the time that Dr. Florey returned from Peoria. Dr. Richards, also an experimentalist of long standing, quickly saw the possibilities inherent in penicillin; he brought to the problem the weight of his influence and his intimate knowledge of the drug houses of this country. To Newton Richards and his Committee on Medical Research must be given the fullest measure of credit for the wise and forceful supervision of the production program.

The later phases of the dramatic story of the antibiotics are related in the papers which follow. Several of these interesting contributions dwell on technical developments; but this must be looked upon as contemporary history, and we therefore deem it appropriate to record here one of the greatest technological achievements of our time.

Antibiosis from Pasteur to Fleming*

JULES BRUNEL†

Je ne juge pas; je raconte.
GOETHE: *Sur la discussion académique entre Cuvier et Geoffroy Saint-Hilaire.*

I. INTRODUCTION

BEING neither a medical man, nor a chemist, nor an engineer, and realizing that practically all those who have had something to do with penicillin belong to one or the other of these professions, I should perhaps relate first how I have become involved in the discussions about the discovery of penicillin, and why I have undertaken this summary of the historical aspects of antibiosis.

Like millions of other non-specialists, I became interested in the antibiosis problem when I read in the August 1943 issue of the *Reader's Digest* a good popular account by J. D. Ratcliff of the "discovery" (so-called) and uses of the "wonder drug," entitled "The Yellow Magic of Penicillin." True, as a professor of mycology, I already had a fairly good idea of the biochemical importance of molds, but Ratcliff's paper was the first intimation I had of the chemotherapeutic value of these lowly plants so often and so long despised. At once I felt that molds were at last vindicated, and that mycology was really coming of age. For a while, I had then an unrestrained admiration for Dr. (now Sir Alexander) Fleming, to whom all credit was unanimously given for that momentous discovery.

However, things soon began to deteriorate. Quite perchance, I found in the bibliography of Charles Thom's splendid monograph, *The Penicillia*,[1] together with the reference to Fleming's original paper of 1929,[2] a reference to a paper in French by André Gratia and Sara Dath entitled "Moisissures et microbes bactériophages." And the date was 1925.[3] I pondered for some time upon that title and that date, and made the following simple reasoning: if, in 1925, somebody knew about molds which could destroy bacteria, Fleming's so-called discovery of 1929 could only be but

* A paper delivered at the 112th meeting of the American Association for the Advancement of Science at St. Louis, Missouri, on 30 March 1946.

† Montreal, Canada.

[1] Thom, C. *The Penicillia*. Baltimore, Williams & Wilkins Co., 1930.

[2] Fleming, A. On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of *B. influenzae*. *Brit. J. exp. Path.*, 1929, *10*, 226–236.

[3] Gratia, André and Dath, Sara. Moisissures et microbes bactériophages. *C. R. Soc. Biol. Paris*, 1925, *92*, 461–462.

another observation of an already known phenomenon. That was the only logical conclusion I could arrive at. So, I went to the library, asked for the *Comptes Rendus de la Société de Biologie de Paris* for 1925, and read with utmost interest Gratia and Dath's paper.

I found that this was the third in a series of short papers on the same general theme presented to the Belgian section of the *Société de Biologie* by André Gratia and various co-workers from the Pasteur Institute in Brussels. The series had begun in 1923 and had continued through 1924, 1925, 1926, and 1931. In 1936 Gratia's work was taken up by Maurice Welsch[4] who published at least twelve or fourteen papers up to World War II and the cessation of intellectual contacts between Belgium and the rest of the world.

After I had gone through the numerous papers of the Belgian school, I decided to read Fleming's paper in the *British Journal of Experimental Pathology*. There, I thought, I would find a full array of bibliographical references to earlier works dealing with antibiosis. To my great surprise, there were only four references: two indifferent ones, and two concerning "pyocyanase."

That seemed to me an impossible situation, and I felt that something should be done about it. At once, I began writing a paper, which I read before the *Société de Biologie de Montréal* (a branch of the Paris Society) on 15 February 1944 and which subsequently appeared in the *Revue Canadienne de Biologie*.[5] Though the circulation of this journal is not very large, my paper reached several important centers, such as Oxford, England, and I soon heard, in more than one way, that it had stirred up interest about the historical aspects of the discovery of penicillin. In that paper I contended that the real discoverer of penicillin was not Fleming and that the observation upon which his so-called discovery rested had been made several times before.

In the autumn of 1945 when Prof. Ernst Chain, the chemist of the group at Oxford studying the antibiotics, came to Montreal, he told me that he knew of a paper published in the 1880's wherein the bacteriostatic properties of a *Micrococcus* were not only described but illustrated with a figure of a culture of anthrax bacillus showing the characteristic sterile zone around the *Micrococcus* colony. After I had written the first half of this paper, I sent a cablegram to Professor Chain asking for the reference to that

[4] The majority of these papers are to be found in the *Comptes Rendus de la Société de Biologie, Paris,* where every volume from *123* (1936) through *131* (1939) contains one or more papers.

[5] Brunel, J. Qui a découvert la pénicilline? *Rev. Canad. Biol.*, 1944, *3*, 333–343. Repr. in *Contributions de l'Institut botanique de l'Université de Montréal*, No. 53.

early paper. He answered that the reference had recently appeared in a paper by Florey in the *British Medical Journal*[6] and that he was sending a reprint by air mail. The reprint was received on 12 March, and the reference was there all right, but I was somewhat disappointed to see that Florey's paper was exactly along the lines of my own, even to the point of mentioning the very quotations from Pasteur and Vuillemin that I had selected to begin my historical account. On the other hand, this coincidence pleased me, because Florey's paper confirmed my assertion that antibiosis dates back seventy years, and that Fleming is but a link in a long chain of workers beginning with none other than Pasteur himself.

It may be thought that I have been, and still am, unduly harsh on a man acclaimed everywhere as a benefactor of mankind, knighted by the King of England, received with great honor by the Pope, bemedalled by scientific societies, and finally made a co-recipient of the Nobel prize in Medicine and Physiology for 1945. But I felt it was my duty to do what I did, and I am confident that more and more people will endorse my point of view when they know the facts, if truth is not a vain word, and if its unbiased search still appeals to many.

May I add that I did not know at first whether Fleming, when he published his celebrated paper of 1929, knew of or had ignored the works of his predecessors. The answer to this was given by Fleming himself in 1945, during the course of an interview with S. J. Woolf of the *New York Times*.[7] Said Sir Alexander Fleming: "I had been working on bacteria ever since my graduation, and accordingly *I was always on the lookout for antibiotics*. So it was perfectly natural that, when I noticed what had happened in the petri dish, I should look for the reason."

"I was always on the lookout for antibiotics." Therefore, we may infer that Fleming *was* familiar with all that had been written on the subject. Why he did not give a full historical summary before reporting his own observations and researches I have no idea.

I had never intended to do any further work on the topic of penicillin or antibiosis after my aforementioned paper was published. As we say in French, "J'avais d'autres chats à fouetter." However, when I received Dr. John Fulton's invitation to participate in a symposium on the history of antibiosis at the St. Louis meeting of the American Association for the Advancement of Science in 1946, I did not hesitate to accept it, because I saw

[6] Florey, H. W. The use of micro-organisms for therapeutic purposes. ***Brit. med. J.*, 1945, 2,** 635–642.

[7] See *The New York Times* Magazine Section, 29 July 1945; also *Amer. Scientist*, October 1945, p. 245.

there an excellent opportunity of discussing the historical aspects of that subject with scientists well versed in it.

After this long preamble, the reader will understand, I hope, why and how I came to write this article, though I never contributed anything to the wealth of experimental facts pertaining to antibiosis.

II. HISTORICAL ASPECTS

As far as I have been able to ascertain, the word "antibiosis" was used for the first time by Dr. Paul Vuillemin in 1889, under the French form "antibiose," in a paper presented to the French Association for the Advancement of Science under the title of "Antibiose et symbiose."[8] The definition given by the author is as follows (I translate):

> An organism destroys the life of another organism to entertain its own life; the former is completely active, the latter completely passive; the active organism is, without restriction, injurious to the life of the passive one. The case is so simple that one has never thought of giving it a name. That condition, instead of presenting itself to our observation in an isolated example, may occur as a factor in more complex phenomena. To simplify the language, we shall therefore call it "antibiosis"; the active individual will be the "antibiont"; the passive one will be the "support." It is exactly the reverse which obtains in *symbiosis*.

If we analyze carefully, word by word, this rather long definition, it becomes evident that the original meaning was far wider than the present one. Compare it, for instance, with the definition given by Waksman on page 271 in his book, *Microbial antagonisms and antibiotic substances*:[9] "*Antibiosis,* the inhibition of growth of one organism by another."

In fact, Vuillemin meant to include in his definition such major misdemeanors as the act of the lion that falls upon its prey, or of the snake that instils venom in its victim's wound before devouring it! This seems a little far-fetched to us, but if we stop to think about the point Vuillemin wanted to prove, his assertion makes sense: he thought that parasitism was always beneficial, in some way, to the parasitized organism. It was a middle term between these two extremes: antibiosis on the one hand, and symbiosis on the other. If the association of two organisms was definitely prejudicial to one of them, we had antibiosis; if it was definitely beneficial to both, we had symbiosis; when in doubt, we had parasitism, which could veer one way or the other, according to circumstances. A more recent way of looking at these interrelations, as Waksman[10] points out, would be:

[8] Vuillemin, P. Antibiose et symbiose. *C. R. Assoc. franç. Avanc. Sci.*, 1889, pt. 2, pp. 525–543.

[9] Waksman, S. A. *Microbial antagonisms and antibiotic substances.* New York, The Commonwealth Fund, 1945.

[10] *Ibid.*, p. 14.

(1) Symbiosis: absolute dependence of one organism upon another.
(2) Metabiosis: one organism of the association is favored by the presence of the other.
(3) Antibiosis (antagonism): one organism of the association is injured by the other.
(4) Parasitism: actual destruction of one organism by another.

Vuillemin in his paper did not mention any case of antibiosis as we understand it today. But he came near our present concept when he wrote: "The simplest case of antibiosis is offered by plants in the dormant stage which offer no resistance whatever to invasion by an enemy." As examples, he cites diseases of potatoes and onions caused by microbes which enter the cells and slowly destroy them; of wheat grains invaded by certain micrococci (caries or smut?) which corrode and kill them.

The fact that Vuillemin coined the word antibiosis does not mean that the phenomenon itself, even as we understand it today, was unknown before him. The mere fact that Pasteur could write in 1877, "La vie empêche la vie," shows that antagonism between living organisms was known to exist at least twelve years before the word antibiosis came into existence. A complete and detailed analysis of the numerous works dealing with antibiosis, from Pasteur to Fleming, would be very long to prepare. Waksman's bibliography of 1945 mentions 1,016 papers, of which 332 (or almost one third) were published in 1929 (the year of publication of Fleming's paper) or earlier. I shall then only present here some significant quotations from a few selected authors.

1. L. Pasteur (1877)—*Bacteria* vs. *Bacteria*

The two following excerpts from Pasteur's memoir of 1877 on "Anthrax and septicemia"[11] will suffice to prove that the conqueror of hydrophobia was already well aware of the phenomenon of antagonism between bacteria:

> Chez les êtres inférieurs, plus encore que dans les grandes espèces animales et végétales, la vie empêche la vie. Un liquide envahi par un ferment organisé ou par un être aérobie permet difficilement la multiplication d'un autre organisme inférieur, alors même que ce liquide, considéré dans son état de pureté, est propre à la nutrition de ce dernier.[12]

[11] Pasteur, L. Charbon et septicémie. *C. R. Acad. Sci. Paris*, 1877, *85*, 101–105. The text of the two quotations is taken from his *Oeuvres*, Paris, 1933, *6*, 177, 178.

[12] "In lower organisms, still more than in the higher animal and plant species, life hinders life. A liquid invaded by an organized ferment or by anaerobic organism hardly allows the multiplication of another lower organism, even though this liquid, considered in its pure state, is favorable to the nutrition of the latter."

L'urine, neutre ou légèrement alcaline, est un excellent terrain de culture pour la bactéridie; que l'urine soit pure et la bactéridie pure, et dans l'intervalle de quelques heures celle-ci est tellement multipliée que les longs filaments qui la composent remplissent le liquide d'un feutrage d'aspect cotonneux; mais si, au moment de déposer dans l'urine les bactéridies à titre de semence, on sème en outre un organisme aérobie, par exemple une des bactéries communes, la bactéridie charbonneuse ne se développe pas ou très peu, et elle périt entièrement après un temps plus ou moins long. Chose bien remarquable, ce même phénomène se passe dans le corps des animaux qui sont le plus aptes à contracter le charbon, et l'on arrive à ce résultat surprenant qu'on peut introduire à profusion dans un animal la bactéridie charbonneuse sans que celui-ci contracte le charbon: il suffit qu'au liquide qui tient en suspension la bactéridie on ait associé en même temps des bactéries communes. *Tous ces faits autorisent peut-être les plus grandes espérances au point de vue thérapeutique.*[13]

Pasteur mentioned another example of antagonism in 1880 when he wrote that he had found "immunity to anthrax obtained in an animal [hen] by means of a totally different parasitic disease [hen cholera]."[14] It would probably be possible to find other instances of definite antagonisms in the seven quarto volumes of Pasteur's collected works. But that would not add to the point I wanted to prove, namely, that this genial man had a clear understanding of a baffling phenomenon, and a real intuition of things to come seventy years later.

And so, let us pass to de Bary.

2. A. DE BARY (1879)

The great German botanist Anton de Bary, according to Waksman,[15] "was the first to emphasize, in 1879, the significance of the antagonistic interrelations among microorganisms; when two organisms are grown on the same substrate, sooner or later one overcomes the other and even kills it. This phenomenon was designated antibiosis." Unfortunately, the article in which de Bary developed this idea, "Die Erscheinung der Symbiose,"[16] was not available in Montreal or in Washington. I cannot therefore give any first-hand information about it. I do not believe, how-

[13] "Neutral or slightly alkaline urine is an excellent medium for the culture of anthrax bacillus; if urine is sterile, and the anthrax bacillus is pure, after a few hours the bacillus has multiplied to such an extent that its long filaments fill the fluid with a downy felt; but if, when inoculating urine with the anthrax bacillus, one inoculates also an aerobic organism, such as one of the "common bacteria," the anthrax bacillus does not grow, or very little, and sooner or later it is entirely destroyed. It is a remarkable thing that the same phenomenon is seen in the body even of those animals most susceptible to anthrax, leading to the astonishing result that anthrax bacteria can be introduced in profusion into an animal, which yet does not develop the disease; it is only necessary to add some "common bacteria" at the same time to the liquid containing the suspension of anthrax bacteria. *These facts perhaps justify the highest hopes for therapeutics.*"

[14] Pasteur, L. Expériences tendant a démontrer que les poules vaccinées pour le choléra sont réfractaires au charbon. *C. R. Acad. Sci. Paris,* 1880, *91,* 315. (*Oeuvres,* Paris, 1933, *6,* 315–316.)

[15] *Op. cit.,* p. 39.

[16] Bary, A. de. *Die Erscheinung der Symbiose.* Strassburg, 1879.

ever, that de Bary ever used the word "antibiosis," which was coined, as I said before, ten years later (in 1889) by Vuillemin.

In 1885, there appeared two significant papers, one in Paris, the other in Naples, on the therapeutic possibilities of bacterial antagonisms.

3. A. V. Cornil and V. Babes (1885)—*Bacteria* vs. *Bacteria*

Cornil and Babes wrote in a French medical journal:[17] "If the study of mutual antagonisms of bacteria were sufficiently far advanced, a disease caused by one bacterium could probably be treated by another." And then: "A further and wider study of this reciprocal action of bacteria may lead to new ideas in therapeutics."

4. A. Cantani (1885)—*Bacteria* vs. *Bacteria*

Cantani, also in 1885,[18] is probably the first to have used an antibiotic procedure in the treatment of an infectious disease, namely, tuberculosis. He wrote: "The known fact that certain bacteria can destroy other, even pathogenic, microbes, if they come in contact with them in any way, gave me the idea of exploring this procedure for the treatment of various infectious diseases." As Florey[19] reports, he then applied what came to be known as "replacement therapy" to a patient suffering from severe tuberculosis: with an atomizer, he insufflated directly into the lungs a culture of what he called *"Bacterium termo"* (probably a mixture of species) mixed with gelatine. The tubercle bacilli disappeared from the sputum and *Bacterium termo* appeared in their place, while at the same time the patient's condition improved.

5. C. Garré (1887)—*Bacteria* vs. *Bacteria*

The Swiss scientist Garré in 1887[20] introduced methods for studying bacterial antagonisms *in vitro* which differ little from those in use today. He inoculated an agar plate with streaks of *Pseudomonas fluorescens* disposed like the spokes of a wheel. After a 24-hour growth period, he inoculated the plate with *Eberthella typhosa* (*Bact. typhosum*) in streaks alternating with those of *Pseudomonas*. Where the two types of bacteria were near one another, that is, in the center, growth of *E. typhosa* was inhibited, while at the periphery colonies were formed to a certain extent.

[17] Cornil, A. V. and Babes, V. Concurrence vitale des bactéries [etc.]. *J. Conn. méd. prat.*, 1885, *7*, 321–323.

[18] Cantani, A. Un tentativo di batterioterapia. *G. int. Sci. med.*, 1885, *n.s. 7*, 493–496.

[19] *Op. cit.*, pp. 635–636.

[20] Garré, C. Ueber Antagonisten unter den Bacterien. *CorrespBl. schweiz. Ärz.*, 1887, *17*, 385–392.

6. E. FREUDENREICH (1888)—*Bacteria* vs. *Bacteria*

Freudenreich in 1888[21] showed that "when certain bacteria were grown in flasks of broth for some time, and then filtered through a porcelain filter, the typhoid germ failed to grow in the filtrate in some cases, and in other filtrates it grew only feebly."

The year 1889 was especially important, because it saw the publication of three papers having a direct bearing upon our subject.

7. P. VUILLEMIN (1889)

Firstly, Vuillemin[22] coined the word "antibiosis" twelve years after Pasteur had discovered the phenomenon. Though Vuillemin gave the word a much broader meaning than it has at present, he should nevertheless be given credit for this philological contribution. When mentioning the word, Waksman refers to two papers, one by Ward[23] (1899) and the other by Behrens[24] (1904), as being the first instances of its use. He does not seem to have known of Vuillemin's paper, as this is not mentioned either in the text or in the bibliography.

8. C. BOUCHARD (1889)—*Bacteria* vs. *Bacteria*

Secondly, a paper by Bouchard[25] entitled "Influence qu'exerce sur la maladie charbonneuse l'inoculation du bacille pyocyanique" indicated that he had "started work on another organism which still continues even at the present time. He published results showing that the inoculation of *Pseudomonas pyocyanea* conferred a considerable degree of protection against *B. anthracis* infection in rabbits, though in guinea-pigs the results were not so good."[26] This led to the isolation of "pyocyanase" ten years later.

9. K. G. DOEHLE (1889)—*Bacteria* vs. *Bacteria*

Thirdly (again in 1889), the German scientist Doehle published a thesis[27] containing the first illustration of antibiotic action: an agar plate of anthrax bacilli, on the surface of which was planted a square of *"Micro-*

[21] Freudenreich, E. De l'antagonisme des bactéries et de l'immunité qu'il confère aux milieux de culture. *Ann. Inst. Pasteur,* 1888, *2,* 200–206.

[22] *Op. cit.* (note 8).

[23] Ward, H. M. Symbiosis. *Ann. Bot., London,* 1899, *13,* 549–562.

[24] Behrens, J. Wechselwirkungen zwischen verschiedenen Organismen (Symbiose, Metabiose, Antagonismus). *Lafars Handb. tech. Mykol. Jena,* 1904, *6,* 2–12.

[25] Bouchard, C. Influence qu'exerce sur la maladie charbonneuse l'inoculation du bacille pyocyanique. *C. R. Acad. Sci. Paris,* 1889, *108,* 713–714.

[26] Florey, *op. cit.,* p. 636.

[27] Doehle, K. G. *Beobachtungen über einen Antagonisten des Milzbrandes.* Kiel, 1889.

coccus anthracotoxicus." Diffusion of an antibiotic from the *Micrococcus* is proven by the sterile zone that surrounds this square colony.

10. B. Gosio (1896)—*Penicillium* vs. *Bacteria*

With regard to *Penicillium* and molds generally, 1896 is a date to remember, because in that year, Gosio,[28,29] an Italian scientist, discovered the first antibiotic produced by a mold of the genus *Penicillium*. This crystalline material, which inhibited the growth of anthrax bacilli, is now called mycophenolic acid. Not having seen Gosio's papers, I mention them on Florey's authority.

11. E. Duchesne (1897)—*Penicillium* vs. *Bacteria*

The following year, the French scientist Duchesne[30] published a thesis in which he reported that "certain green *Penicillia* are capable of repressing the growth of various bacteria or of bringing about their attenuation." Waksman, from whom I have quoted the lines above, writes that Duchesne is the first to have reported antibacterial properties in molds. He seems to ignore Gosio's name and work, mentioned by Florey, which antedates Duchesne by one year. All this shows how difficult it is to establish, definitely and for all time, priority in matters which have been worked out by so many men at about the same period.

12. R. Emmerich & O. Löw (1899)—*Bacteria* vs. *Bacteria*

The German workers Emmerich and Löw[31] in 1899 introduced the first antibacterial extract into medicine. This extract, prepared from old cultures of *Pseudomonas pyocyanea,* was called pyocyanase. Emmerich and Löw showed that it was capable of lysing suspensions of *B. anthracis in vitro* in a short time, and was also bactericidal to *Bact. typhosum* (*Eberthella typhosa*), *Corynebacterium diphtheriae, Staphylococcus* and *Pasteurella pestis.*

An abundant literature accumulated on the subject of pyocyanase, which was often tried in therapeutics, both by injection or as a local anti-

[28] Gosio, Bartolomeo. Ricerche batteriologische e chimiche sulle alterazioni del mais. Contributo all'etiologia della pellagra. (Memoria 2a) *Riv. Igiene San't. pubbl.*, 1896, *7*, 825–849.

[29] Gosio, Bartolomeo, and Ferrati, E. Sull' azione fisiologica dei veleni del mais invaso de alcuni ifomiceti. Contributo all'etiologia della pellagra. (Memoria 3a.) *Ibid.*, 1896, *7*, 961–981.

[30] Duchesne, E. Contribution à l'étude de la concurrence vitale chez les microorganismes; antagonisme entre les moisissures et les microbes. (Thesis) Lyon, 1897.

[31] Emmerich, R., and Löw, O. Bakteriologische Enzyme als Ursache der erworbenen Immunität und die Heilung von Infektionskrankheiten durch dieselben. *Z. Hyg. Immun.*, 1899, *31*, 1–65.

septic. But gradually enthusiasm died out, and by 1913 Sonnenberger deplored the fact that "papers on therapy with pyocyanase have almost disappeared from recent literature."

13. W. D. FROST (1904)—*Bacteria* vs. *Bacteria*

According to Frost,[32] an American bacteriologist who published an important paper on bacterial antagonism in 1904, "*Eberthella typhosa* can be antagonized by a number of different soil bacteria, of which *Pseudomonas fluorescens* exhibits the strongest effect." Among other techniques devised by Frost, there is that of the collodion membrane interposed between a culture of an antibiont and a culture of another organism to be antagonized, the idea being that the antibiotic could diffuse through the membrane while the organisms themselves could not pass from one culture medium to the other.

14. A. STURLI (1908)—*Penicillium* vs. *Bacteria*

Next in line is a little-known author, Adriano Sturli, seldom if ever mentioned in the historical accounts of antibiosis. Neither Waksman nor Florey so much as allude to him. Nevertheless, he made observations and experiments, published in 1908, that bear directly upon our subject. My attention was drawn to Sturli's paper by its title alone: "Ueber ein in Schimmelpilzen (*Penicillium glaucum*) vorkommendes Gift."[33] While perusing this article, I came upon the following sentence: "Wegen der geringen Menge der rein dargestellten Substanz wurde ihre toxische Wirkung nur auf Bakterien geprüft (Wachstumshemmung einer Anthraxkultur im Löfflerschen Bouillon durch Zusatz von 0.05 g. der Substanz)."[34]

When I published my paper of 1944, Sturli's article was the earliest reference I had to a paper dealing unquestionably with an antibiotic produced by a *Penicillium*. But I wrote then: "It is not only possible but very likely that an earlier reference will be found." These earlier references I subsequently discovered were Duchesne (1897) and Gosio (1896), already mentioned.

15. R. WESTLING (1912)

I should like to digress here to say a few words about a scientist whose

[32] Frost, W. D. The antagonism exhibited by certain saprophytic bacteria against *Bacillus typhosus* Gaffky. *J. infect. Dis.*, 1904, *1*, 599–640.

[33] Sturli, Adriano. Ueber ein in Schimmelpilzen (*Penicillium glaucum*) vorkommendes Gift. *Wien. klin. Wschr.*, 1908, *21*, 711–714.

[34] "Owing to the small quantity of the pure extract, its toxicity could be assayed only with Bacteria; it brought about inhibition of growth, in a culture of anthrax bacillus on Löffler's bouillon, through addition of five centigrams of the substance."

name is practically never mentioned in connection with penicillin or *Penicillium*. Everybody knows that penicillin is produced chiefly by a definite species of *Penicillium* called *Penicillium notatum*. But nobody seems to be concerned as to who has named and described this most important plant. Such a lack of interest is not peculiar to this one case. On the contrary, it seems to be the usual attitude of microbiologists toward the names of the biological entities with which they have to deal day after day. They are names, and that is all there is to it.

Botanists are usually more careful about giving due credit to authors of plant names, and in this instance they would say that the full name of the *Penicillium* is *Penicillium notatum* Westling, because this remarkable species was first understood, named, and described by Westling.[35] True, this systematician had not the faintest idea of the fame which the new plant would one day attain, but his merit still remains, just as that of Vuillemin who coined "antibiosis" and of Fleming who coined "penicillin."

I do not mean to say that *Penicillium notatum* should never be mentioned without the name of Westling following it. But in titles or sub-titles of books and papers, authors should see to it that Westling's name is mentioned, just as that of Thom should appear after *Penicillium chrysogenum*, the other penicillin-producing species, and that of Link after *Penicillium* as a genus. After all, think what confusion would exist in this world if the two billion individuals that people the earth had no distinctive names of their own. The same is true of the animal and plant kingdoms, and we should never forget that those scientists who have striven to give us a clear and understandable picture of the living world deserve their share of gratitude when a particular species they have named attains universal distinction.

16. A. VAUDREMER (1913)—*Aspergillus* vs. *Bacteria*

In 1913 Vaudremer,[36] in France, found that filtrates of *Aspergillus fumigatus* could destroy the tubercle bacillus. This is particularly interesting because it has since been shown that *A. fumigatus* produces four antibiotics: spinulosin, fumigatin, fumigacin, and gliotoxin. This mold is therefore one of the most important species at the present time; but let us not forget that its use began sixteen years before publication of Fleming's first paper on penicillin.

In view of the fact that so much attention has been given to the search

[35] Westling, R. Über die grünen Spezies der Gattung Penicillium. *Ark. Bot.*, 1912, *11*, 1–156.

[36] Vaudremer, A. Action de l'extrait filtré d'Aspergillus fumigatus sur les bacilles tuberculeux. *C. R. Soc. Biol. Paris*, 1913, *74*, 278–280, 752–754.

for the perfect antibiotic with which to fight tuberculosis, it is well to recall these words of Vaudremer, which might have been written yesterday: "From the point of view of the treatment of tuberculosis in man any conclusion is premature. Since 1910 we have treated more than 200 patients with extracts of *Aspergillus fumigatus* in several hospitals and sanatoria in Paris. From the cases we have observed it is justifiable to conclude that the injections, which have never caused any febrile reaction, are harmless. Sometimes healing which we had not hoped for has taken place unexpectedly. In other cases there has been transient improvement, but unfortunately, there are still many instances in which the tuberculous process continues to take its course."

An excellent summary of Vaudremer's pioneer investigations on antibiosis which he pursued from 1892 to 1927 is contained in a paper by Guilhon.[37] It also contains a good portrait of that brilliant bacteriologist and clinician.

17. R. LIESKE (1921)—*Actinomycetes* vs. *Bacteria*

The actinomycetes are a group of micro-organisms which may be said to be on the border between bacteria and fungi. As early as 1890 Gasperini[38] had already shown that certain species of *Streptomyces* brought about the lysis of various micro-organisms.

Lieske, who published a monograph on the actinomycetes in 1921,[39] "demonstrated that specific actinomycetes are able to bring about the lysis of many dead and living bacterial cells; they are selective in their action, affecting only certain bacteria such as *S. aureus* and *S. pyogenes,* but not *S. lutea, S. marcescens,* or *Ps. aeruginosa.*"[40] Lieske, however, did not carry out any therapeutic applications of these interesting facts.

18. A. GRATIA (1923–1939)

A couple of years later, the Belgian scientist Gratia and his collaborators from the Pasteur Institute in Brussels began publishing an extensive series of short papers, mainly in the *Comptes Rendus de la Société de Biologie de Paris.* I have already mentioned in my introduction that one of these papers[41] was the starting-point of my investigations in the history of anti-

[37] Guilhon, J. Les mycoïnes ou fongines et les métabolites antimicrobiens. *L'Encyclopédie Vétérinaire périodique,* 1945, fasc. 9–10, pp. 296–320.

[38] Gasperini, G. Recherches morphologiques et biologiques sur un micro-organisme de l'atmosphère, le Streptothrix Foersteri Cohn. *Ann. Microgr.,* 1890, *10,* 449–474.

[39] Lieske, R. *Morphologie und Biologie der Strahlenpilze.* Leipzig, 1921.

[40] Waksman, *op. cit.,* p. 104.

[41] See note 3.

biosis. As the work of that Belgian school is very extensive and covers a period of more than fifteen years (1923–1939); as, furthermore, it displays a remarkable parallelism with that of the English scientists of London and Oxford; as, finally, it has provoked a good deal of controversy, I have studied it in a more detailed manner and shall deal more thoroughly with it.

Gratia and his co-workers were chiefly concerned with the bacteriolytic properties of actinomycetes (*Streptothrix* or *Actinomyces*) rather than of real molds. However, in their third paper (1925), Gratia and Dath[42] could write: "From a contaminate culture of anthrax, completely clarified, we have isolated a variety of *Penicillium glaucum* dissolving the anthrax bacillus." And in the same paper, one finds the following significant statement: "The lysis caused by *Streptothrix* operates through a very active and eminently diffusible agent that one finds again, separate from the mold, in the dissolved and filtrated microbial emulsions."

In a later paper of 1925,[43] they write that Lieske had already observed the antibiotic properties of actinomycetes, and very candidly they add: "We want to mention ourselves the priority of Lieske in regard to a phenomenon which we had thought we were the first to observe."

Now, all this was sufficient to bring doubt into my mind when I first heard of the discovery of penicillin, though the whole world was then clamoring that for the first time in history a powerful antibiotic substance had been obtained from a lowly green mold, an ordinary *Penicillium* such as everybody has seen on oranges or strawberry jams.

Whatever opinion we may hold now about the relative importance of "actinomycetin" (as it was called later by Welsch) and penicillin, and even though penicillin later proved to be more potent and more practical than actinomycetin as a chemotherapeutic agent, I think it cannot be denied that "Gratia's substance" was used clinically long before penicillin, not only, as Florey asserts, for immunization purposes, but for real therapeutic purposes.

Gratia[44] wrote in 1930 that results obtained "in chronic staphylococcal infections resistant to all treatments, show that mycolysates must have antigenic properties." And he adds: "We have sought mycolysis of other pathogenic bacteria which had not yet been obtained. We have thus, more than a year ago, easily obtained the mycolysis of *Streptococcus,* as well as

[42] See note 3.

[43] Gratia, A. and Dath, S. A propos de l'action bactériolytique du Streptothrix. *C. R. Soc. Biol. Paris*, 1925, *93*, 451.

[44] Gratia, A. Le traitement des infections à staphylocoques par le bactériophage et les mycolysats staphylococciques. *Bull. Soc. nat. Chir.*, 1930, *56*, 344–348.

that of *Pneumococcus,* diphtheria and whooping-cough bacilli. Jaumain has obtained that of *Gonococcus.*"

And for those who assert, *a priori,* that the few antibiotics discovered before penicillin were all toxic for the animal, and consequently of no value in therapeutics, I shall add this other observation of Gratia: "All these mycolysates, some of which, as that of diphtheria for example, come from highly toxic microbes, have been administered at high doses, for long periods, to guinea-pigs and rabbits, without any accident whatever." This, I think, proves not only that the bacteria were completely destroyed, but that the lytic substance from the mold was absolutely harmless to the animals.

And again, for those who should still doubt that the mycolysates of the Belgian biologist have ever been used on man, I shall quote the following case, from which it is not easy to draw conclusions as to the therapeutic value of the mycolysate used, because it was not used *alone,* but which proves definitely the non-toxicity of "Gratia's substance" for man:

> A patient who, for three years, had not spent a single day without a boil, in spite of all treatments applied by several physicians, was sent to us by a despairing dermatologist. Jaumain, after having made three injections of bacteriophage, did not hesitate to pursue the treatment with a series of injections of staphylococcal mycolysate.
>
> The result was remarkable; the cure was not only quickly achieved, but lasting, as there was not the slightest relapse since it was effected three years ago.
>
> Since then, we have applied *in a large number of cases* this association of bacteriophage and staphylococcal mycolysates, and we believe that it is to-day the most efficacious treatment for the most stubborn staphylococcal infections; it is quick and sure; it presents no danger; it is not painful and is easy to apply.

These lines were not written, as you may think, in 1943 or 1944, but in 1930. Remember that it was only in 1941 that similar results were obtained with penicillin!

19. A. FLEMING (1929)—*Penicillium* vs. *Bacteria*

Such was the situation in 1929, when Alexander Fleming published the paper[45] in which he announced that he had discovered an antibiotic produced by a *Penicillium.* He did not know the real nature of that substance and so he wrote: "In the rest of this article allusion will constantly be made to experiments with filtrates of a broth culture of this mould, so for convenience and to avoid the repetition of the rather cumbersome phrase 'mould broth filtrate,' the name 'penicillin' will be used."

Fleming's paper was forgotten for ten years, and penicillin was never used for therapeutic purposes until the Oxford group, under Howard

[45] *Op. cit.* (note 2).

Florey, took up the problem, and Ernst Chain, the chemist of the team, devised a method of extraction and obtained in 1940, under a stable form, pure penicillin as we have it today.

The rest is current history.

III. Conclusions

To conclude this historical account, I think it is evident that antibiosis, which has received so much attention in the last decade, is an old question, and that many workers have contributed to its progress. The study of antibiotics culminated in the isolation of penicillin in 1940 and in its widespread use as a chemotherapeutic agent. The subject as a whole received then, but only then, a distinct impetus. This led many people to believe that antibiosis dates back only to 1940, or at the earliest to 1929.

As Dr. John F. Fulton wrote:[46] "The development of penicillin . . . lacks the element of a clear-cut and isolated discovery for which full credit should be given to one man." We should not be too much surprised at this, because such is often the way discoveries are made, by the long-continued process of addition of bits of information. And in this particular case, it is still less surprising because empirical knowledge long preceded scientific knowledge. In my own province of Quebec, I have been told that in Charlevoix County old people long used molds on jams to take care of their respiratory ailments: they simply stirred mold and jam together and took a spoonful of the remedy! In central Europe, it is a well-known fact that farmers always kept a molded loaf of bread to prevent infection: "When any member of the family received an injury such as a cut or bruise, a thin slice from the outside of the loaf was cut off, mixed into a paste with water and applied to the wound with a bandage. No infection would then result from such a cut." (A. E. Cliffe, personal communication.) There is evidence also that the North American Indians, and even the Mayans, had some knowledge of the curative power of molds.

Without defending empiricism as a system, all this should make us a little more humble and more historically conscious. Men of science should never forget the extraordinary adventure that befell the great American naturalist Louis Agassiz: While studying the habits of a family of fish, the Siluridae, he thought he had discovered something entirely new, but before publishing anything about it, he delved into the literature and finally found that his "discovery" had already been made by a naturalist whose name was . . . Aristotle!

[46] Fulton, J. F. Penicillin, plasma fractionation, and the physician. *Atlantic Monthly*, 1945, *176*, 107–114.

The Work on Penicillin at Oxford

H. W. FLOREY and E. P. ABRAHAM*

GREAT interest in what the popular press called the "story of penicillin" was aroused by the introduction of the drug into therapeutics in the early 1940's. The story contained certain elements of the dramatic, so that both medical and lay writers have been tempted to treat simple historical facts in a somewhat imaginative manner. A detailed account of the history of penicillin can now be found in a recent publication (*Antibiotics*, Florey, Chain, Heatley, Jennings, Sanders, Abraham, and Florey, London, 1949, Chapters 15 and 20).† This description has been used as a basis for the shorter account given here of the work done at Oxford during the war.

The simplest and clearest way to consider the introduction of penicillin into medicine is to view the work as having been done in two distinct parts, separated by time and carried out by two independent sets of investigators working in different places. The first phase consisted in the observations on the existence of penicillin and on some of its properties by Fleming, beginning in 1928 and apparently ceasing about 1932 except for some observations on its use in bacteriological media. This work was done at St. Mary's Hospital, Paddington, London. Into this phase falls the work of Clutterbuck, Lovell, and Raistrick at the London School of Hygiene and Tropical Medicine. They took the first steps in the isolation of penicillin but abandoned the work owing to the instability of the active substance.

Following these events there was a gap of some years during which the possibility of using penicillin as an antiseptic, as had been suggested by Fleming, passed out of mind.

During the later 1930's work had been pursued on lysozyme at the Sir William Dunn School of Pathology, Oxford, which was a continuation of work started some years before at Sheffield. From this work grew the project of an investigation of antibacterial substances of microbic origin already described in the literature, such for instance as penicillin, and the products of *B. subtilis* and *Ps. pyocyanea*. For the proposed investigation Chain and Florey made an application for funds to the Rockefeller Foundation in November, 1939.

The work to which reference was made in the application had been started in 1938 by Chain and Falk with a culture of Fleming's strain of

* Oxford, England.

† Part of the text of this paper has been taken directly from Chapter 15.

Penicillium notatum which had been maintained for some years in the Sir William Dunn School of Pathology, having been obtained from the National Collection of Type Cultures by Dreyer and Campbell-Renton for use in research on bacteriophage. They performed some experiments with it, but at this time energy was chiefly being applied to the study of the action of lysozyme, and it was not until late in 1939 that work on penicillin was taken up vigorously by Chain, Florey, and Heatley.

The crude testing methods first employed were improved by a new method of assay, subsequently known as the cylinder and plate (or cylinder-plate) method, which greatly simplified the chemical work.

Various media were tested and the modified Czapek-Dox medium used by Clutterbuck, Lovell, and Raistrick, and attributed by them to Dr. H. G. Turley, was adopted as the basis for production. A number of adjuvants, such as marmite, malt extract, peptic and tryptic meat digests, peptone, meat extracts, and yeast extract were tested at different concentrations and sometimes in combination. Changes in the nature and amount of the constituents of the basic medium were also made. These experiments, which continued for several months and were interspersed among other work, were not made systematically, and with the limited assay facilities available only marked differences in titre were noticed. The penicillin yield was depressed or abolished by some variations, but a significant, repeatable increase in titre was not obtained. From an early stage of the work (1939) the medium was made up with one tenth of its volume of a boiled brewer's yeast extract because, although this had little or no effect on the ultimate titre, it stimulated the growth of the fungus and the maximum yield of penicillin was obtained two to three days earlier than in its absence.

As soon as active culture fluid was available a preliminary study of the chemical properties of penicillin and of methods of extracting it was begun by Chain and Heatley. They confirmed and extended the observations of Clutterbuck, Lovell, and Raistrick that penicillin was an acid of low molecular weight, soluble and stable in various solvents, but stable in water only in the form of its salts at a pH between 5 and 8. Penicillin could thus be extracted from the culture fluid after acidification to pH 2 into organic solvents in the form of the free acid, and from the organic solvent back into water in the form of its salts by the addition of alkali to bring the pH of the aqueous phase to neutrality, and these operations could be carried out without appreciable loss of activity if the solutions were kept at a temperature between $+2°$ and $+5°$ C. At room temperature the penicillin

solutions lost their activity rapidly at pH values below 4 and above 9. Attempts to obtain penicillin salts in a dry state showed that aqueous penicillin solutions could be evaporated to dryness *in vacuo* from the frozen state without loss of activity, and eventually a small amount of hygroscopic brown powder, containing the sodium salt of penicillin, was obtained by the procedure outlined. The brown powder containing the sodium salt of penicillin retained its activity for some time when kept dry. Its antibacterial power was high when compared with that of other antibacterial agents such as the acridine derivatives and the sulphonamides, as it inhibited the growth of staphylococci at a dilution of 1 in 500,000. At that time the enormous power of pure penicillin was not realized and it was thought possible, in view of the great antibacterial strength of the brown powder, that the sodium salt of penicillin had been obtained in a fairly high degree of purity. In actual fact subsequent investigations showed that the preparation had contained less than 1 per cent of penicillin. However, under the impression that the material was fairly pure, tests of toxicity were carried out which showed that the intravenous injection of doses as large as 10 mg. was tolerated by mice weighing 20 g. without much sign of toxic effect. It must be considered extremely fortunate that the first preparations of the sodium salt of penicillin, which although of great antibacterial activity contained over 99 per cent of impurities, showed so little toxicity. If even one of the many impurities had been highly toxic, as it well might have been, the non-toxicity of penicillin might have been completely masked, with unpredictable effects on subsequent work. The next step was to see whether the preparations retained their antibacterial activity *in vivo* and were capable of effectively protecting mice from experimental infections. It was on 25 May 1940 that the first attempt was made to see if an extract exerted any protective effect on infected mice. The mice were infected intraperitoneally with a strain of haemolytic streptococcus and divided into three groups. One group (A) consisted of two mice, each of which received subcutaneously 5 doses of 0.1 ml. of a solution containing 50 mg. of penicillin powder per ml. 1, 3¼, 5¼, 7⅓, and 11 hours after infection; the second group (B) consisted of two mice which received one dose of 0.2 ml. of the same solution 1 hour after infection. Four mice in a third group served as controls. The penicillin extract used probably contained between 2 and 4 units per mg. The results were that of group A, one mouse survived till the 13th day and the other indefinitely; of group B, one survived for 2 and the other for 6 days. All the control mice were dead within 16½ hours.

This experiment, imperfect as it was, sufficed to give grounds for the

hope that penicillin would have some systemic chemotherapeutic properties so that it was clear that further investigation should be carried out with as great a speed as possible. N. G. Heatley devoted his attention to the production of penicillin while the collaboration of other workers secured a wider examination of the other problems involved. A. D. Gardner, who had already contributed valuable advice, actively took up the bacteriological investigations and had the assistance of J. Orr-Ewing for studies on the anaerobic bacteria. M. A. Jennings was responsible for some bacteriological work and with H. W. Florey undertook the pharmacological and biological investigations. The group was joined by A. G. Sanders, who later set up and operated one of the large-scale extraction plants, and by E. P. Abraham, who collaborated with E. Chain on the chemical and biochemical aspects of the work. When the stage of clinical trials was reached, H. W. Florey, first with C. M. Fletcher and later with M. E. Florey, was responsible for the first clinical work. Thus the group of workers, now sometimes referred to as the Oxford team, was gradually assembled and their work was greatly facilitated by the devoted labours of the following technical assistants: J. B. Burtt, D. and R. Callow, E. Cooke, S. A. Cresswell, P. Gardiner, G. Glister, R. Holt, C. Inayat, J. Kent, M. Lancaster, P. McKegney, and E. Vincent. The work was much accelerated by the most intimate collaboration of all concerned and the success attained was undoubtedly due to the combined efforts of the members of the group.

The mouse protection experiments were repeated with larger numbers of animals and greater quantities of penicillin and showed clearly that penicillin acted as a systemic chemotherapeutic agent against experimental infection with *Str. pyogenes, Staph. aureus,* and *Cl. septicum*. They also threw light on the quantities of penicillin required for protection, and on methods of administration.

It is necessary to understand this term "systemic chemotherapeutic agent" if the reason for the interest aroused by the work is to be appreciated. When used in reference to the treatment of microbial infection the term "systemic chemotherapeutic agent" means a substance which when given "systemically", i.e., so that it permeates through the whole or most of the body, endows the circulating blood and the tissue fluids with the power to stop the growth of infecting micro-organisms. To do this it must have various properties, but chiefly it must combine high antimicrobial power with low toxicity to the body tissues. This point may be clearer if the results obtained with tyrothricin, which was extracted and described by Dubos and Cattaneo in 1939, are considered. Tyrothricin is a powerful

antibacterial substance, but its toxicity to animals is such that it can only be used for application locally to infected areas—it is harmful if put into the blood stream. It is not therefore a systemic chemotherapeutic agent.

It was, then, the experiments showing the protection of mice from infection which were crucial for the "development" of penicillin into the product with a wide range of clinical uses that has now become familiar.

Following these experiments, additions to knowledge of the behaviour of penicillin accentuated its possible advantages over the sulphonamides, which had been introduced into medicine in the middle 1930's. Among the weaknesses of the sulphonamides were the facts that they were to a large extent inactivated by pus and other breakdown products of tissues, that their efficacy appeared to be inversely proportional to the number of bacteria present, and that they not infrequently produced toxic symptoms when given in adequate doses. It was therefore of great importance that penicillin was active in the presence of pus, blood, serum, and tissue autolysates, and that the number of bacteria present appeared to affect its action very little. Moreover as purer and purer preparations of penicillin became available, it was shown that the toxicity of preparations decreased as their antibacterial potency increased.

In the early work it soon became apparent that it was necessary to express the antibacterial activity of culture fluids and extracts in terms of some unit. A buffer solution of partially purified penicillin was therefore selected, the activity in 1 ml. of which was defined as 1 unit. This unit was transferred to other standard preparations, liquid and later dry, and became known as the Oxford unit. In October 1944 at a meeting held in London under the auspices of the League of Nations Health Organisation, the International Penicillin Unit, now in universal use, was fixed at substantially the same value as this unit.

The gratifying results of the first mouse protection experiments led to the immediate realization of the possible therapeutic value of penicillin in man, especially in the war-wounded, and it was at once decided to explore every possible means of obtaining enough of the material for preliminary trials of the drug in infections in man. The enthusiasm of the Oxford workers was believed by many to be premature, and though an approach was made to a commercial firm, this firm and others who might otherwise have helped in the project were overburdened with war work. It was shortly afterwards, too, that the worst period of enemy bombing began in England (September, 1940).

It was therefore decided to try to prepare the material in the laboratory

in Oxford, which fortunately suffered no interference from enemy activity, on a scale large enough to make clinical trials possible. At that time the best titre obtained in the metabolism liquor was about 2 units per ml., and the best final recovery after extraction was seldom as high as 40 per cent. Thus the preparation of one million units for clinical use required at least 1,500 litres (400 U.S. gallons) of crude culture fluid, entailing the cultivation of 1,000 square feet of mycelium. For an ordinary research institute, cultivation on this scale, as well as the labour of extracting and concentrating the active substance, was formidable. It was made more difficult by the fact that however large the scale, the cultures had to be kept throughout under the most stringent aseptic conditions.

The experiments on mice had been done with material grown in Erlenmeyer flasks and extracted by hand in separating funnels. But conical flasks were wasteful of autoclave and incubator space and in any case were not available in sufficient number. A flat vessel was needed in which a large area of mycelium could grow on a layer of fluid not more than 2 cm. deep. Various kinds of domestic enamelware trays, dishes and pans fitted with lids, and biscuit and other tins (both with and without an internal coating of lacquer) were tried. The old-style circular bed-pan with a spout proved to be much the most satisfactory, but the demand for these for the military hospitals made them impossible to obtain in quantity. By early 1941 the culture vessel difficulty was completely and expeditiously solved by Messrs. James Macintyre & Co., of Burslem, to whom we were introduced by Dr. P. J. Stock. They made specially for the purpose a rectangular ceramic slipware vessel measuring 27.5 x 22 x 6 cm. with a sloping spout 3 cm. in diameter, and holding one litre of fluid in a layer 1.7 cm. deep. These vessels could be stacked horizontally in the incubator with great economy of space, and could be filled and autoclaved in a vertical position. They were inoculated with a spore suspension and incubated at 24° C. in the laboratory operating theatre (already fitted with thermostatically controlled heating), being stacked on discarded bookcases from the Old Bodleian Library. The maximum titre was reached after six to seven days, when the medium was harvested and fresh medium was run under the mycelium with full aseptic precautions. This and subsequent replacements reached the same titre in only three to four days. The harvesting was partly mechanised and the replacement technique was used for some months but was then abandoned because the contamination rate from a troublesome spore-bearing organism became too high.

At first, extraction was done by hand in improvised separating funnels

consisting of inverted one-gallon bottles, bored with a small hole to admit air, which could be closed by a screw. To minimise destruction during the water-to-solvent extractions when penicillin was in the labile form of the free acid, they were carried out either under cramped conditions in the cold store or, when the weather was sufficiently wintry, on the roof of the laboratory. The discomfort of these wearying and protracted sessions stimulated the construction of a semi-automatic extraction apparatus which could be used at room temperature. It worked on the counter-current principle, fine droplets of acidified penicillin being allowed to fall through a column of slowly ascending amyl acetate. Several difficulties had to be overcome, and when a workable model had been made it required a good deal of maintenance and attention. It was superseded after a year by a simpler plant of larger capacity, but meanwhile it had processed nearly all the penicillin used for the first trials on man.

FIRST CLINICAL TRIALS

The first patient was given an intravenous injection on 27 January 1941. The administration of 10 mg. of the material had caused no observable symptoms in a mouse so that it was considered safe to give 100 mg. to the patient. Although there was no immediate reaction to the injection the patient had a rigor about three hours later. A repetition of the experiment on another patient left no doubt that the substance was pyrogenic. Once this phenomenon had been observed in man it was easy to show that injection of the material caused a sharp rise of temperature in the rabbit. Fortunately, it was shown that by means of chromatography on alumina the pyrogenic substance could be eliminated and that penicillin itself was not the cause of the trouble.

Enough material was then collected to treat some cases of severe infection. Most of it contained from 30 to 50 units per mg., though some had a potency as low as 4.8 units and some as high as 110 units per mg. These preparations though exceedingly impure—the best not containing more than 7 per cent of pure penicillin—gave satisfactory clinical results as the impurities had no significant pharmacological effects.

All the first cases treated were severe, and treatment in most cases was only permitted after all other available means of treatment had failed. Five of the first six patients had staphylococcal or streptococcal infections. On 12 February 1941 treatment was begun at the Radcliffe Infirmary, Oxford, of the first patient, who had a severe staphylococcal infection with osteomyelitis and abscess formation. After five days' treatment, during which

considerable clinical improvement had occurred, the stock of penicillin was exhausted and the patient relapsed and eventually died. The next case, though not ending with death, demonstrated that five days' treatment was also insufficient to eliminate streptococci. The third patient gave a strikingly successful response, his large carbuncle of the back disappearing without surgical intervention. This patient, incidentally, was the husband of one of the cleaners in the Sir William Dunn School of Pathology. The fourth patient was a boy with cavernous sinus thrombosis who when treatment began was considered to be moribund. Penicillin was administered for nine days, and subsequently the child seemed to be well on the way to recovery when unfortunately a mycotic aneurysm of the vertebral artery ruptured and caused death. The fifth case was a boy with staphylococcal septicaemia and osteomyelitis. He received continuous intravenous penicillin and after ten days' infusion and four days of intermittent injections recovered fully with an excellent functional result. A sixth case, a baby with a staphylococcal infection of the urine, was cured by oral administration of the drug.

The results of the treatment of these cases, and a full description of the method of preparation, the pharmacological and bacteriological properties of penicillin were published in August 1941.

It was realized that further progress depended on the assistance of commercial undertakings of considerable size. At the time that the clinical observations were being made it seemed very unlikely that adequate help could be obtained in England. A visit from Dr. Weaver of the Natural Sciences Division of the Rockefeller Foundation offered opportunity to put to him the possibility of something being done in the U.S.A. As a result of this Florey and Heatley went to the United States in June 1941. They arrived just before the celebrations of 4 July, and spent the first few days with Dr. J. F. Fulton at New Haven. After the situation had been explained to him he suggested that they see Dr. Ross Harrison, Chairman of the National Research Council, who advised them to talk to Dr. Thom of the Bureau of Plant Industry at Beltsville, Maryland. Dr. Thom took them to see Dr. Wells at the Department of Agriculture in Washington, and after a very friendly discussion it was agreed that they should go to the Northern Regional Research Laboratory of the Department of Agriculture at Peoria. The problem of producing more penicillin was put to the Director of the Laboratory, Dr. May, and to the Director of the Fermentation Division, Dr. Coghill. It is worth noting, in view of subsequent developments, that at that first meeting on 14 July 1941 Coghill

asked whether deep fermentation on the lines of that used for the production of gluconic acid had been tried and suggested that this might be the key to successful commercial production. Florey then visited a number of drug firms in the United States and Canada with a request which now looks modest but at the time seemed formidable—to brew 10,000 litres of culture fluid and extract the penicillin, so that more clinical trials might be made at Oxford. None of the information which had up till then been accumulated at Oxford was withheld. Though certain of the firms thought the matter worth attention, a number of them showed little interest, and some none at all. Amongst the first to tackle the problem seriously were Merck & Co., Inc., E. R. Squibb & Sons, and Chas. Pfizer & Co., Inc. It is not proposed here to pursue further this link of American work with that of Oxford. An account of subsequent developments will be found in the reference already given.

As far as securing supplies for further clinical work at Oxford went, this expedition bore little fruit, so that the Oxford workers were thrown back after all on their own resources and the small but willing help of Imperial Chemical Industries Ltd., who supplied small amounts of dry product, and of Messrs. Kemball, Bishop & Co. Ltd., who sent a large number of 150-200 gallon lots of crude active culture fluid to Oxford by road to be processed there.

With material obtained in this way a second series of cases was treated in Oxford, beginning in January 1942. The patients were treated systemically and by local application. They served to confirm fully the favourable results of the first series. These results were published by Florey and Florey in March 1943.

In the meantime the first case had been treated in the United States in March 1942, and in August 1943 a report was published of 500 cases treated by parenteral administration. The results concurred with those obtained in Oxford and this concurrence was striking in that they were the pooled observations of 22 groups using widely different doses and procedures. The doses recommended in the U.S.A. agreed very closely with those that had been found satisfactory in Oxford—the Americans recommended at least 10,000 units every two to three hours, while the Oxford recommendation was 15,000 units three-hourly.

It was evident at an early date that penicillin might prove valuable for the treatment of war-wounded if enough of it could be produced. The work begun at Oxford was continued into the Army. In April 1942 a small quantity of penicillin prepared in Oxford was offered to Major-General Poole, Director of Pathology, War Office, and was dispatched to the Mid-

dle East. This material, which contained 30 to 40 units per milligramme, was used by Pulvertaft for local application to wounds which had long been suppurating, some of them for many months. The results, which could be described as encouraging in spite of the character of the cases, were published by Pulvertaft in 1943, though reports had been circulated privately before this. Other very small amounts were later sent to Cairo to continue treatment of the chronically infected. By the beginning of 1943 sufficient supplies had been accumulated from Imperial Chemical Industries Ltd., Messrs. Kemball, Bishop & Co., Ltd., and Oxford to make it worth while for the Army to send special investigators to North Africa. For this purpose Ian Fraser, a surgeon, and Scott Thomson, a bacteriologist, after preliminary special training at Oxford, were sent in May 1943 to Algiers. About three weeks later H. W. Florey, and a month after that H. Cairns, the neurosurgeon, professor of surgery at Oxford, also went to North Africa.

Thus with the help of surgeons experienced in war surgery the foundations were laid for the successful use of penicillin for the wounded. The film made by Florey of some of the first cases treated at Tripoli remains, as far as the authors are aware, the only cinema film made of the early methods of treatment and of the results obtained in the British Army. A report on the work in Africa was published in October 1943 and the film was used for instructional purposes.

By the end of 1943, as far as Oxford was concerned, the main interest had moved to the endeavours to elucidate the chemical structure of the drug, though of course chemical investigations had been continuing from an early date. Work on other antibiotics was pursued and as a consequence of a survey of the products of *Penicillia* and *Aspergilli* it was found that many species produced penicillin-like substances. As a result of this work and similar observations elsewhere it is now known that at least 23 species of *Penicillium* and 7 species of *Aspergillus* produce such substances.

Chemical Investigations

After the favourable results of the first therapeutic experiments in mice, further work on the isolation of penicillin was begun by Chain and Abraham at the Sir William Dunn School of Pathology, and this work went on in parallel with the biological investigations. Very small amounts of the drug were present in the culture fluids available at that time, and the isolation of pure material was a much more formidable task than it is today.

Penicillin was found to be rapidly inactivated in aqueous solution by

acid, alkali, heat, oxidising agents, various heavy metal ions, and an enzyme from bacteria called penicillinase. It followed that only mild procedures could be used for its purification, and attention was concentrated on distribution between water and organic solvents and on chromatography. Some of the early preparations obtained after chromatography on alumina, which had an activity of about 50 units per mg., were used in the first clinical trials in man in 1941. It was then found that further impurities were removed, by adsorption on alumina, when crude aqueous solutions of penicillin were treated with aluminium amalgam, and a year later material was obtained in the form of a barium salt which had an activity of nearly 700 units per mg. This material inhibited the growth of *Staph. aureus* at a dilution of 1 in more than 24 millions. Inactivation by cold acid or alkali was shown to result in the liberation of new titratable groups and hydrolysis under more drastic conditions to cause the evolution of carbon dioxide.

When material of such high activity had been prepared it seemed worth while to begin to study its chemical nature, and in this work Chain and Abraham were joined by Robinson and Baker, and later by Cornforth, of the Dyson Perrins Laboratory, Oxford. The material was shown to be nitrogenous, but owing to an error by an analyst it was thought to be sulphur-free. Although it was eventually found to contain not more than 50 per cent of penicillin, degradation products were obtained from it which proved to be of fundamental importance in elucidating the structure of the active molecule. The characterisation of minute amounts of crystalline degradation products was facilitated by Crowfoot and Low in the Oxford University Department of Crystallography, who made X-ray crystallographic measurements, and by Holiday, working in the Department of Biochemistry, who determined ultra-violet absorption spectra.

Other groups, both in England and America, were now beginning to work in the field. They included Cook and Heilbron at the Imperial College of Science and Technology, London, Duffin and Smith at the Wellcome Foundation, and E. R. Squibb & Sons, Merck & Co., Inc., Chas. Pfizer & Co., Inc., and the Northern Regional Research Laboratory of the U.S. Department of Agriculture. As chemical information accumulated it began to appear conceivable that penicillin would be obtained synthetically before many months had passed, and that its production in large amounts might then present relatively little difficulty. In view of the importance of the drug to war medicine the Lord President of the Council imposed a ban on the publication of the results of the chemical work in Great Britain

early in 1943, and publication was also withheld in the U.S.A. Exchange of information between British and American chemists at first took place through private channels and later through Committees of the Medical Research Council in London and the Office of Scientific Research and Development in Washington. The American group of investigators was soon enlarged by the inclusion of more industrial and academic research laboratories, and the various groups entered into contracts with their governmental agency. Although the hope of producing synthetic penicillin was not in fact fulfilled, the imposition of secrecy was clearly in the Anglo-American interest at the time, and the arrangement for keeping the various workers informed of each other's progress worked well until it was suspended at the end of 1945.

During 1943 much progress was made both with the purification of penicillin and with the investigations into its structure. The discovery that sulphur was present in the molecule was made simultaneously at Oxford and at the Squibb Institute for Medical Research. The use of the newly described method of partition chromatography on silica-gel enabled preparations of penicillin to be obtained at Oxford and Imperial Chemical Industries Ltd. with an activity of more than 1,000 units per mg. At the same time highly active preparations were being obtained in the U.S.A., and a crystalline sodium salt of penicillin was first isolated by Wintersteiner and MacPhillamy at the Squibb Institute for Medical Research. Soon afterwards the sodium salt of material prepared at Oxford was found to crystallise.

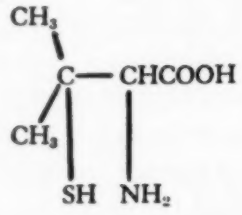
At this stage it became apparent that the American and British penicillins were not identical. The former had the molecular formula $C_{16}H_{18}O_4N_2S$ and yielded phenylacetic acid on hydrolysis, while the latter had the formula $C_{14}H_{20}O_4N_2S$ and yielded no phenylacetic acid. The British substance was subsequently called penicillin I (or F) and the American penicillin II (or G). Several other penicillins were later identified. Penicillin III (or X) was isolated at the Northern Regional Research Laboratory, Peoria, in 1944, and a fourth substance, penicillin K, was obtained at the Abbott Laboratories in 1945.

Penicillamine, an important constituent of all the penicillins, was first obtained at Oxford by hydrolysis of preparations of penicillin I with hot acid. It was later shown to be β-thiolvaline (I) and to belong to the D-series of amino acids.

The identification of penicillamine and carbon dioxide as products of hydrolysis of penicillin I left eight of its fourteen carbon atoms to be

accounted for. These carbon atoms were found in an aldehyde with formula $C_8H_{13}O_2N$ which was isolated at Oxford in the form of a dinitrophenyl-

(I)



hydrazone. The aldehyde, called penillo-1-aldehyde, remained in solution after penicillin had been hydrolysed with hot acid or cold alkali and the penicillamine precipitated by the addition of mercuric chloride. It proved to be a hexenoylaminoacetaldehyde (II)

(II) $CH_3CH_2CH{=}CHCH_2CONHCH_2CHO$

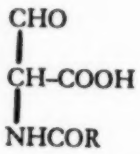
This work showed that the structure of penicillin I could be obtained by the appropriate condensation of penicillamine, penillo-1-aldehyde, and carbon dioxide. The results of parallel work in the U.S.A. made it clear that penicillin II could be constructed from penicillamine, carbon dioxide, and phenylacetylaminoacetaldehyde (III)

(III) $C_6H_5CH_2CONHCH_2O$

Investigations at the Imperial College of Science and Technology in London showed that the penicillin studied there could be degraded to yield n-caproylaminoacetylaldehyde and that it was therefore dihydropenicillin-I.

The ease with which carbon dioxide was eliminated from the penicillin molecule suggested that it came from a carboxyl group in the β-position to the carbonyl group of the aldehyde fragment. Before carbon dioxide was lost the aldehyde could thus be assigned the structure (IV) in which R depended on the nature of the penicillin.

(IV)



The product obtained by inactivating penicillin with dilute alkali had the properties of a thiazolidine, for it broke down into penicillamine, an aldehyde and carbon dioxide on the addition of mercuric chloride. It could

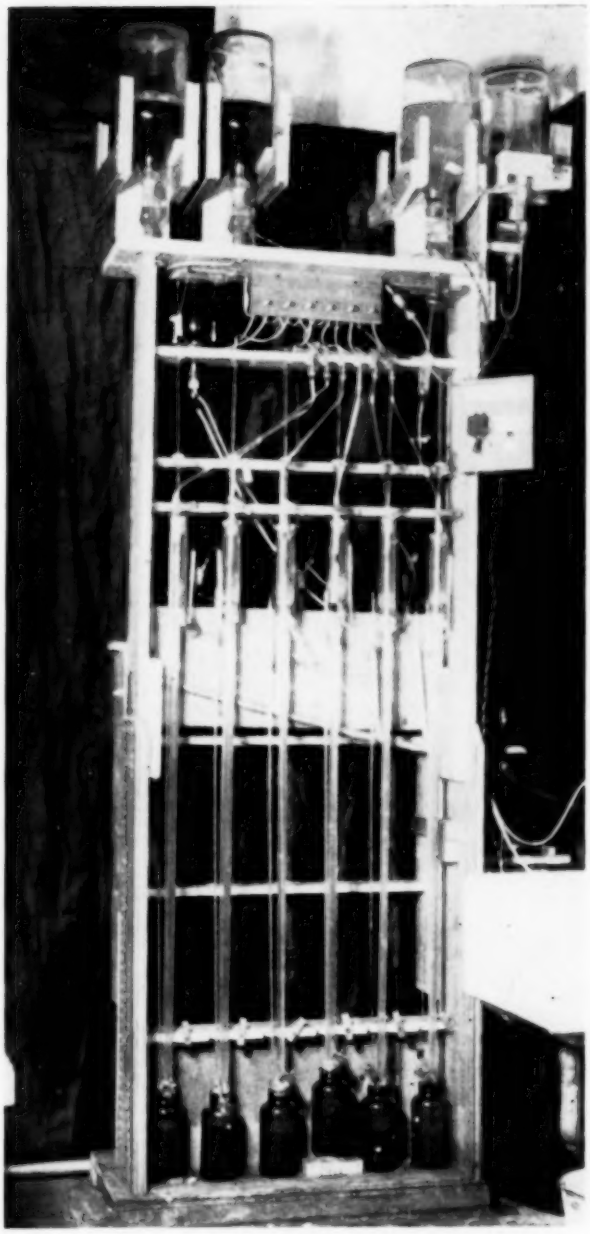


Fig. 1. The first semi-automatic extraction apparatus. Uniform droplets of acidified penicillin fell counter-current through slowly rising columns of amyl acetate to which they gave up their penicillin. (Reproduced from Florey *et al.*, *Antibiotics*, with kind permission of the Oxford University Press.)

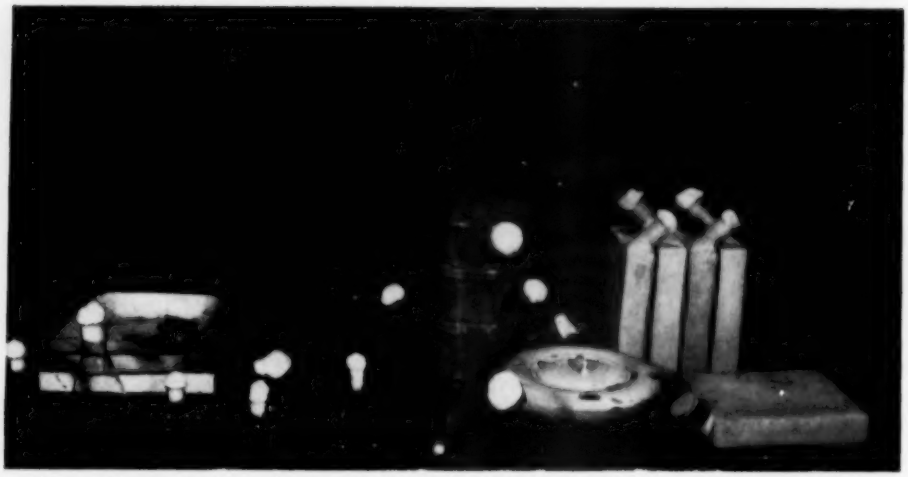


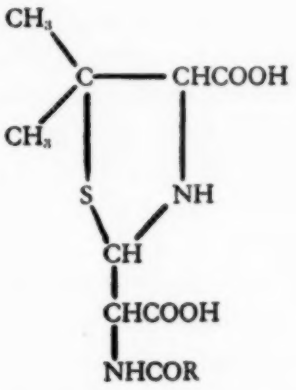
Fig. 2. Some of the culture vessels first tried for penicillin production. The specially made rectangular ceramic vessels are shown at the right next to the bedpan, of which they were the logical development.

Fig. 3. Horizontally stacked rectangular vessels, each containing one litre of sterile medium, being inoculated with a suspension of spores of *Penicillium notatum*. (Reproduced from Florey *et al.*, *Antibiotics*, with kind permission of the Oxford University Press.)

Fig. 5. Part of the second extraction plant showing milk cooler and churns in which cooled culture fluid, amyl acetate, and acid were emulsified. (Reproduced from Florey *et al., Antibiotics,* with kind permission of the Oxford University Press.)

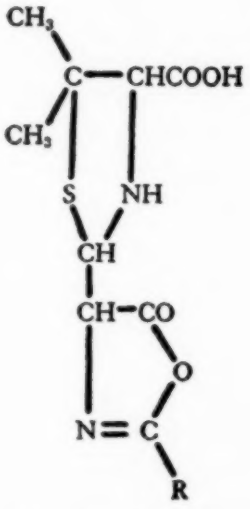
therefore be assigned the structure (V), a structure that was later established beyond doubt in the Merck Laboratories. The structure of penicillin would then be found by the elimination of one molecule of water in an appropriate manner from (V).

(V)

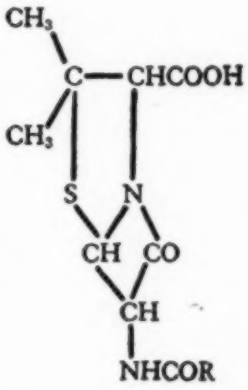


On the basis of these and other considerations the Oxford workers suggested the thiazolidine-oxazolone structure (VI) or the β-lactam structure (VII) for penicillin I ($R=C_5H_9$) in October 1943, and in November the Merck group proposed essentially the same formulae ($R=C_6H_5CH_2$) for penicillin II. The thiazolidine-oxazolone formula was proposed independently by the Squibb and Abbott groups.

(VI)



(VII)



Although other formulae were later suggested, only (VI) and (VII) survived the accumulation of further evidence. The decision between them

proved remarkably difficult. Two important new degradation products of penicillin were isolated by the Merck chemists in 1944, but one of these products contained an oxazolone ring and the other a β-lactam ring. The absence of a basic group in penicillin was readily accounted for by the β-lactam structure, but arguments were developed to support the claim that the nitrogen in the thiazolidine ring of structure (VI) would also be non-basic. In the spring of 1945, however, the results of a detailed X-ray crystallographic analysis carried out by Crowfoot and Low at Oxford, and Bunn and Turner-Jones at Imperial Chemical Industries Ltd., showed conclusively that the crystalline alkali-metal salts of penicillin had the β-lactam structure (VII).

By the middle of 1943 it was already clear that penicillin was not a very complex molecule, and during 1944 and 1945 many groups in the U.S.A. and England were trying to synthesise it. The result of this intensive work was unexpectedly disappointing, for no satisfactory synthesis was achieved. Nevertheless, the failure was not complete. The Merck group and the Oxford workers, by condensing penicillamine with various oxazolones, obtained products which showed an activity of about 1 unit per mg. In one case good evidence was obtained that the activity was due to the formation of a minute amount of penicillin, since the product was inactivated by treatment with the enzyme penicillinase. The nature of the active product was finally proved by the Cornell group in 1946, who succeeded in isolating from it a small quantity of the crystalline trimethylamine salt of penicillin II.

By 1951 a satisfactory synthesis of penicillin was still unaccomplished. Nevertheless, the chemical investigations of this substance have revealed much of fundamental importance. Penicillin contains a β-lactam grouping hitherto unknown in nature, and its chemical reactivity is in some ways as remarkable as its biological properties. The work which began in a small way at Oxford and culminated in the investigations of many groups of chemists in England and America added an interesting chapter to organic chemistry.

We are indebted to Dr. M. A. Jennings and Dr. N. G. Heatley for assistance in the preparation of this manuscript. Those interested in further details relating to the introduction of penicillin into medicine are referred to Chapters 15 and 20 of *Antibiotics,* where full bibliographical data are given.

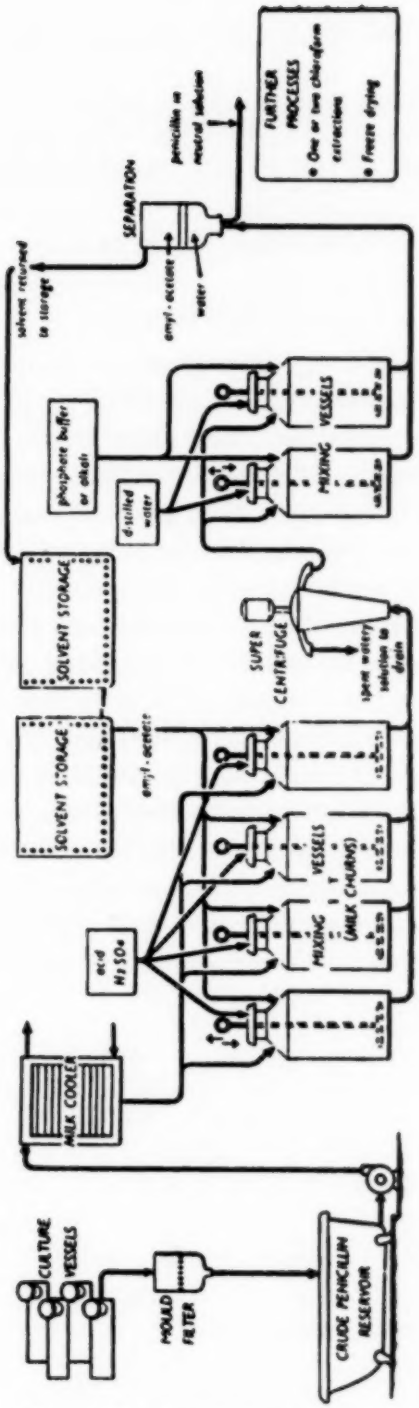


Fig. 4. The flow-sheet of the second extraction plant built at Oxford. (Reproduced from E. S. Duthie, *Molecules Against Microbes*, with kind permission of Sigma Books Ltd.)

Streptomycin
Isolation, Properties, and Utilization*

SELMAN A. WAKSMAN†

Introduction

STREPTOMYCIN belongs to a group of compounds produced by micro-organisms, possessing antimicrobial properties in dilute solutions, and known as antibiotics. These differ greatly from ordinary antiseptics and disinfectants in their chemical nature, antimicrobial properties, and effect upon the animal body. They are highly selective in their action upon different micro-organisms, this specificity being known as the antibiotic spectrum. This selective action is both qualitative and quantitative in nature. Although it has been known since the latter part of last century that certain micro-organisms are capable of producing substances which prevent the growth of and even destroy pathogenic bacteria, it is only within the last decade that antibiotics have begun to find extensive application as chemotherapeutic agents. Among the first antibiotics which have found such application, penicillin and streptomycin occupy a prominent place. Penicillin is largely active against gram-positive bacteria, certain gram-negative cocci, anærobic bacteria, and spirochætes. Streptomycin is also active against a variety of gram-negative and acid-fast bacteria, as well as against some of the gram-positive organisms which have become resistant

Table 1

Antibacterial activities of several antibiotics

Minimum concentration required to inhibit growth, in micrograms per milliliter

	Gliotoxin	Penicillic acid	Penicillin G	Streptothricin	Streptomycin
Bacillus mycoides	0.25	32	30.00	100.0	0.13
Bacillus subtilis	0.25	8	0.03	0.8	0.25
Staphylococcus aureus	0.15	16	0.016	0.1	0.03
Escherichia coli	25.00	64	14.00	0.3	0.25
Klebsiella pneumoniae	6.00	64	110.00	0.1	0.13
Pseudomonas aeruginosa	500.00	1,000	500.00	2.0	4.00
Mycobacterium phlei	4.00	64	14.00	7.0	0.25
Mycobacterium smegmatis	4.00	32	450.00	14.0	1.00

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to penicillin (Table 1). Neither penicillin nor streptomycin is active against viruses and fungi. Some of the more recently isolated antibiotics are highly active against these microbial forms.

Like penicillin preceding it, streptomycin has in a phenomenally brief period come to occupy an important place in the history of medical science. *Streptomyces griseus,* the organism producing streptomycin, was first isolated in this country in 1915, but it was not tested at that time for its antimicrobial properties. The strain producing streptomycin was first isolated in September 1943 in the laboratories of the Department of Microbiology of the New Jersey Agricultural Experiment Station, Rutgers University. The first public announcement of its isolation was made in January 1944. Before the end of 1944, streptomycin was already being tested for clinical effects, and within two years an extensive amount of investigational work, comprising microbiological, chemical, pharmacological, and clinical studies, was accomplished; the practical potentialities of the drug were thereby definitely established.

In 1945, it gradually came to be recognized that streptomycin is highly effective against the tuberculosis organism, not only *in vitro* but also *in vivo.* Several centers were soon established for the testing of the sensitivity of different freshly isolated strains of *Mycobacterium tuberculosis* to streptomycin and for the practical evaluation of this antibiotic in experimental animals. Following the lead of the Mayo Clinic, the American Trudeau Society, the National Tuberculosis Association, and the Veterans Administration took an active part in these investigations. Early in 1947, a conference was arranged for the discussion of the application of streptomycin to clinical tuberculosis, methods of administration, and possible toxic effects.

Within three years after the announcement of streptomycin, its chemistry was completely elucidated, the first chemical derivative (dihydrostreptomycin) was obtained, and the first thousand clinical cases treated with streptomycin were reported. From a laboratory curiosity, streptomycin production gradually grew into a large industry, with a monthly yield of nearly 10,000 kilograms in the United States alone. A number of factories have also been established abroad.

This rapid progress in the development of streptomycin was due largely to two factors. First, the spectacular recognition of penicillin and its utilization for disease control between 1941 and 1943 suggested the possibility of finding other antibiotics which might be effective in the treatment of diseases not affected by penicillin. Secondly, knowledge of the actinomycetes already existed in the Department of Microbiology at

Rutgers, dating back nearly three decades, and this led in 1940 to the isolation of actinomycin and in 1942 to the isolation of streptothricin—a substance which paved the way for the isolation of streptomycin and which resembles it both chemically and biologically.

The Committee on Chemotherapeutics of the National Research Council contributed considerably to the co-ordination of the clinical work on streptomycin. At first, all streptomycin produced was reported to the Civilian Production Administration. Allocations were made to the Army, Navy, U. S. Public Health Service, Veterans Administration, and the National Research Council, after adjustment of the available supply to their needs. No other agency was allowed to purchase streptomycin. No patient who received it paid for it, and no physician was charged for it. The program of clinical research was conducted by the concerted efforts of the government, manufacturers, National Research Council, and civilian medical scientists, for the sole purpose of obtaining the necessary information concerning streptomycin in the shortest possible time.

This comprehensive research program on streptomycin constituted the first privately financed, nationally co-ordinated clinical evaluation in history. It was made possible by a joint contribution of nearly a million dollars from pharmaceutical and chemical manufacturers who produced streptomycin. It was estimated that in October 1946 the manufacturing laboratories had invested twenty million dollars in production facilities. About 1,600 general hospitals were designated at that time as depots for the drug. Any non-depot hospital or any physician could call upon a depot hospital for the purchase of streptomycin for the treatment of streptomycin-indicated conditions. The requests of other institutions were given careful consideration. Each depot hospital was limited to a monthly quota. At the beginning of 1947, the Committee on Chemotherapy completed its work. Enough streptomycin was soon produced to make possible wide sales and even export abroad, the export amounting in 1949 to the phenomenal figure of more than forty million dollars. The price dropped from twenty dollars a gram in 1946 to about thirty-two cents in 1950.

PRODUCTION OF ANTIBIOTICS BY ACTINOMYCETES

The isolation of streptomycin was the culminating point of a long and painstaking search for antimicrobial agents produced by actinomycetes, a group of organisms closely related to the bacteria on the one hand, and to the fungi on the other, and widely distributed in nature. Streptomycin is produced by certain strains of *Streptomyces griseus,* one of the species of

the actinomycetes. These organisms comprise 20 per cent or more of all the colonies developing on an ordinary bacteriological agar plate from a given suspension of soil in water. A large percentage of these actinomycetes are capable of inhibiting the growth of various bacteria, fungi, certain viruses, and virus-like organisms.

The ability of actinomycetes to inhibit the growth of micro-organisms has been known for a long time. Lieske[1] had shown in 1921 that certain forms are capable of bringing about lysis of many bacteria. This process was selective in nature, since certain bacteria were affected and others were not. Antibiotic-like substances were demonstrated, prior to 1940, for actinomycetes in only two instances. One of these was a preparation studied first by Gratia and Dath[2] in 1925 which had the capacity to lyse dead typhoid cells and certain living bacteria; this preparation was later designated by Welsch[3] as actinomycetin. Several Russian investigators made a comprehensive study of the distribution, among actinomycetes, of the capacity to antagonize the growth of various bacteria; one preparation was described as actinomyces lysozyme. Nakhimovskaia[4] found that antagonistic actinomycetes are widely distributed in nature: out of 80 cultures isolated from several soils, 47 possessed antagonistic properties, but only 27 liberated active substances into the medium; these possessed the capacity of inhibiting the growth of gram-positive bacteria, but not of gram-negative bacteria or fungi.

A comprehensive survey of the distribution of antagonistic properties among actinomycetes was made in the Microbiology Department of the New Jersey Agricultural Experiment Station in 1940.[5] Out of 244 cultures freshly isolated from the soil, 106, or 43.4 per cent, possessed some effect upon bacteria, and 49 cultures, or 20 per cent, were highly active. A similar proportion was obtained in a study of the various cultures kept in the laboratory collection: out of a total of 164 cultures examined, 33 per cent had an activity against *S. aureus,* and 14.6 per cent had a marked effect. The nature of the test organism, the composition of medium, and the method of testing were found to influence greatly the results obtained. In another survey of 187 freshly isolated cultures, only 3 per cent gave a

[1] Lieske, R. *Morphologie und Biologie der Strahlenpilze.* Leipzig, G. Borntraeger, 1921.

[2] Gratia, A., and Dath, S. De l'action bactériolytique du *Streptothrix. C. R. Soc. Biol. Paris,* 1923–1926, *91,* 1442–1443; *92,* 461, 1125–1126; *93,* 451; *94,* 1267.

[3] Welsch, M. Bacteriostatic and bacteriolytic properties of actinomycetes. *J. Bact.,* 1942, *44,* 571–588; 1947, *18,* 1–315.

[4] Nakhimovskaia, M. The antagonism between actinomycetes and soil bacteria. *Microbiol.* (U.S.S.R.), 1937, *6,* 131–157.

[5] Waksman, S. A., Horning, E. S., Welsch, M., and Woodruff, H. B. Distribution of antagonistic actinomycetes in nature. *Soil Sci.,* 1942, *54,* 281–296.

high activity against *E. coli* when tested on nutrient agar and 6 per cent on dextrose-asparagine agar. The corresponding figures for those active against *B. subtilis* were 16 and 44 per cent.

The first antibiotic produced by an actinomyces was isolated in the Department of Microbiology in 1940. This substance was designated as *actinomycin* and the organism producing it as *Actinomyces antibioticus.*[6] The substance was crystallized and its chemical and biological properties were established. It proved to be extremely toxic to the animal body, thus eliminating its chemotherapeutic potentialities.

These studies were continued and later yielded streptothricin,[7] streptomycin,[8] grisein, streptocin, neomycin, fradicin, and certain others. Of these streptomycin proved to be most effective in clinical therapy. Meanwhile, the search was begun in other laboratories and yielded such important compounds as chloromycetin, aureomycin, and terramycin.

At present, more than sixty antibiotics are known to be produced by actinomycetes (Table 2). They differ markedly from the antibiotics pro-

TABLE 2

Classification of Antibiotics Produced by Actinomycetes

A. Soluble in ether and in other organic solvents:
  I. Pigmented, largely antibacterial, substances:
    1. *Actinomycin* A, B, C, J_2
    2. *Xanthomycin* A, B
    3. *Xanthomycin-like* substances
    4. *Pikromycin*
    5. *Actinoflavin*
    6. *Litmocidin*
    7. *Actinorhodin*
    8. *Nocardin*
    9. *Actinomycelin*
    10. *Rhodomycin*
    11. *Rhodomycetin*
  II. Non-pigmented or yellowish-colored substances:
    IIa. Active largely against bacteria or bacteria and rickettsiae:
      1. *Proactinomycin*
      2. *Mycomycin*
      3. *Chloromycetin*
      4. *Aureomycin*

[6] Waksman, S. A., and Woodruff, H. B. *Actinomyces antibioticus*, a new soil organism antagonistic to pathogenic and non-pathogenic bacteria. *J. Bact.*, 1941, *42*, 231–249.

[7] Waksman, S. A., and Woodruff, H. B. Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against gram-negative bacteria. *Proc. Soc. exp. Biol., N.Y.*, 1942, *49*, 207–210.

[8] Schatz, A., Bugie, E., and Waksman, S. A. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Proc. Soc. exp. Biol., N.Y.*, 1944, *55*, 66–69.

5. *Terramycin*
6. *Flavomycin*
7. *Luteomycin*
8. *Monamycin*

IIb. Active against fungi:

1. *Actidione*
2. *Antimycin*
3. *Thiolutin*

IIc. Active largely against protozoan organisms:

1. *Streptocin*
2. *Borrelidin*

B. Insoluble in ether, but soluble in other organic solvents:

I. Violet-blue pigmented substance:

1. *Mycetin*

II. Colorless, sulphur-containing substance:

1. *Sulphactin*

III. Colorless, nitrogenous body, active against fungi:

1. *Fradicin*
2. *Musarin*

IV. Extracted from mycelium:

1. *Micromonosporin*
2. *Fungicidin*

C. Soluble in water, insoluble in ether and in other organic solvents:

I. Bases soluble in aqueous acid solution, removed from charcoal by acid alcohol:

Ia. Streptothricin and streptothricin-like substances:

1. *Streptothricin*
2. *Streptin*
3. *Streptolin*
4. *Lavendulin*
5. *Actinorubin*
6. *Antibiotic* 136
7. *Streptothricin* VI
8. *Netropsin*
9. *Aureothricin*
10. *Roseomycin*

Ib. Streptomycin and streptomycin-like substances:

1. *Streptomycin*
2. *Mannosidostreptomycin*
3. *Dihydrostreptomycin*
4. *Hydroxystreptomycin*

Ic. Neomycin complex:

1. *Neomycin*
2. *Neomycin* A
3. *Neomycin* B, C

Id. Other basic substances:

1. *Viomycin*
2. *Reticulin*
3. *Dextromycin*

II. Removed from charcoal by neutral alcohol:
1. *Grisein*
2. *Grisein-like* substances (3510)

D. Proteins and polypeptides:
I. Colourless preparations, possessing lytic properties against living gram-positive bacteria and dead gram-negative bacteria:
1. *Actinomycetin*
2. *Actinozyme*
II. Active largely against micrococci; lysis of cell membrane:
1. *Actinomyces lysozyme*

E. Incompletely described agents:
I. Active against the smegma bacillus:
1. *Smegmatis factor*
II. Active against bacteriophages:
1. *Antiphage factors*
III. Active against viruses:
1. *Antiviral antibiotics*

F. Agents not produced readily in liquid media; activity obtained only on agar streak:
I. Little known substances:
1. *Insoluble factors*

G. Combinations of several antibiotics:
I. *Aureotracin,* a combination of aureomycin and bacitracin

duced by fungi and bacteria. Of particular significance are the characteristic properties, designated as antibiotic spectra or specific activity upon different micro-organisms. This is brought out in Table 1.

Each of these antibiotics may be produced by several species of actinomycetes. Actinomycin, for example, is formed by *Streptomyces antibioticus* and by a large number of other species of *Streptomyces;* some of these give rise to various chemical modifications of the actinomycin molecule, which have been designated as A, B, and C. Different forms of streptomycin are produced by *S. griseus, S. bikiniensis,* and *S. griseocarneus;* under certain conditions of culture, various mixtures of streptomycin and mannosido-streptomycin are formed by the first, whereas the last organism gives rise to hydroxystreptomycin. Some organisms may form mixtures of different antibiotics, such as streptomycin and streptothricin or neomycin and fradicin. *S. griseus* itself, in addition to the two forms of streptomycin, also produces actidione and streptocin.

ISOLATION OF STREPTOTHRICIN

Streptothricin was the first antibiotic which was isolated from a culture of an actinomyces and which appeared to show distinct promise as a

desirable chemotherapeutic agent, since it was active against both gram-positive and gram-negative bacteria and was not very toxic to animals. It was obtained from a culture of an organism isolated in the laboratories of the Microbiology Department in 1941. This organism was identical with *Actinomyces lavendulae,* a culture isolated from the soil in the same laboratory twenty-five years previously. Streptothricin possessed highly desirable physical, chemical, and antibacterial properties, and offered promise of filling the gap left by tyrothricin and penicillin, since the latter were largely active against gram-positive bacteria and cocci. Streptothricin was a basic compound, soluble in water, with an optimum activity at slight alkalinity. It was effective, both *in vitro* and *in vivo,* against a number of bacteria and upon fungi. It was resistant to heat and to the action of different micro-organisms and enzymes.[9]

When a study of the pharmacology of streptothricin was undertaken, it was found to exert a residual toxic effect upon the animal body.[10,11] Its use for parenteral administration was, therefore, definitely excluded. Its potential practical application would have to be limited to oral or topical administration.

Discovery of Streptomycin

The experience gained in the study of the production and isolation of streptothricin proved to be highly significant in the planning of a further search for antibiotics which would possess chemical and biological properties similar to it but which would be less toxic to the animal body. It was clear that cultures of organisms producing such substances could be obtained on continued screening, since the necessary methods for their preliminary testing, their cultivation on suitable media (both in stationary and in shaken conditions), and the isolation of the antibiotics produced by them were now well recognized. It was, of course, desirable that the antibiotic spectrum of any new substances should be broader than that of streptothricin, i.e., be more active against a greater variety of those bacteria which are usually not very sensitive to penicillin. After an examination of many freshly isolated cultures, representing various species of actinomycetes, an organism was obtained which was capable of yielding a desirable antibiotic. This was designated as streptomycin. The organism producing

[9] Waksman, S. A. Production and activity of streptothricin. *J. Bact.,* 1943, *46,* 299–310.

[10] Metzger, H. J., Waksman, S. A., and Pugh, L. H. *In vivo* activity of streptothricin against *Brucella abortus. Proc. Soc. exp. Biol., N.Y.,* 1942, *51,* 251–252.

[11] Robinson, H. J. Some toxicological, bacteriological, and pharmacological properties of antimicrobial agents produced by soil microorganisms. Thesis. New Brunswick, N.J., Rutgers University, 1943.

the new agent was very similar to one well known in the laboratory and isolated from soil in 1916. It was described at that time as *Actinomyces griseus;*[12] in view of the fact that the generic name of this group of actinomycetes had been changed early in 1943 from *Actinomyces* to *Streptomyces,*[13] the new culture would automatically be designated as *Streptomyces griseus,* thus accounting for the name of the new substance. Although several additional cultures of streptomycin-producing strains of *S. griseus* were isolated later, one of the 1943 strains has been largely utilized for the commercial production of streptomycin (Fig. 1).

The medium finally adopted for the production of streptomycin was different from that used for streptothricin. It contained a source of nitrogen (peptone), carbon (glucose), certain growth-promoting substances (meat extract), mineral salts (NaCl), and tap water. It was later found that casein hydrolyzate or soy bean meal could be substituted for peptone, yeast extract or corn steep liquor could replace meat extract, and Fe, Mg, and K salts could take the place of the minerals in the water. The culture had to be well aerated and incubated at 25° to 30°C. for 2 to 5 days.[14]

Under suitable conditions, the activity of the culture medium reached 100 to 200 micrograms per milliliter of streptomycin. In commercial production, much higher activity is usually obtained, ranging from 500 to more than 1,000 micrograms per milliliter of culture. The medium is either inoculated with spore material; or, for large-scale production, pregerminated spores or mycelial growth thus obtained after 24 or 40 hours shaking of spore suspension are used for inoculation purposes. Several consecutive transfers may be made before large tanks are finally inoculated, so as to shorten the period of incubation. For the preservation of the culture, either inoculation into sterile soil or lyophilyzing may be resorted to.

The actual manufacture of streptomycin consists of the following four stages: (i) fermentation of the culture, (ii) recovery of the crude material, (iii) chemical purification of the streptomycin, and (iv) finishing process. Raw materials of the nutrient medium are mixed with water in a concentration about ten times that finally required, and the mixture is stored until needed. It is pumped into the fermenters and properly diluted; the mash is then sterilized by heating at approximately 120°C., cooled, and inoculated with spores or mycelial growth of *S. griseus.*

[12] Waksman, S. A., and Curtis, R. The actinomyces of the soil. *Soil Sci.,* 1916, *1,* 99–134.

[13] Waksman, S. A., and Henrici, A. T. The nomenclature and classification of the actinomycetes. *J. Bact.,* 1943, *46,* 337–341.

[14] Waksman, S. A., Bugie, E., and Schatz, A. Isolation of antibiotic substances from soil microorganisms with special reference to streptothricin and streptomycin. *Proc. Mayo Clin.,* 1944, *19,* 537–548.

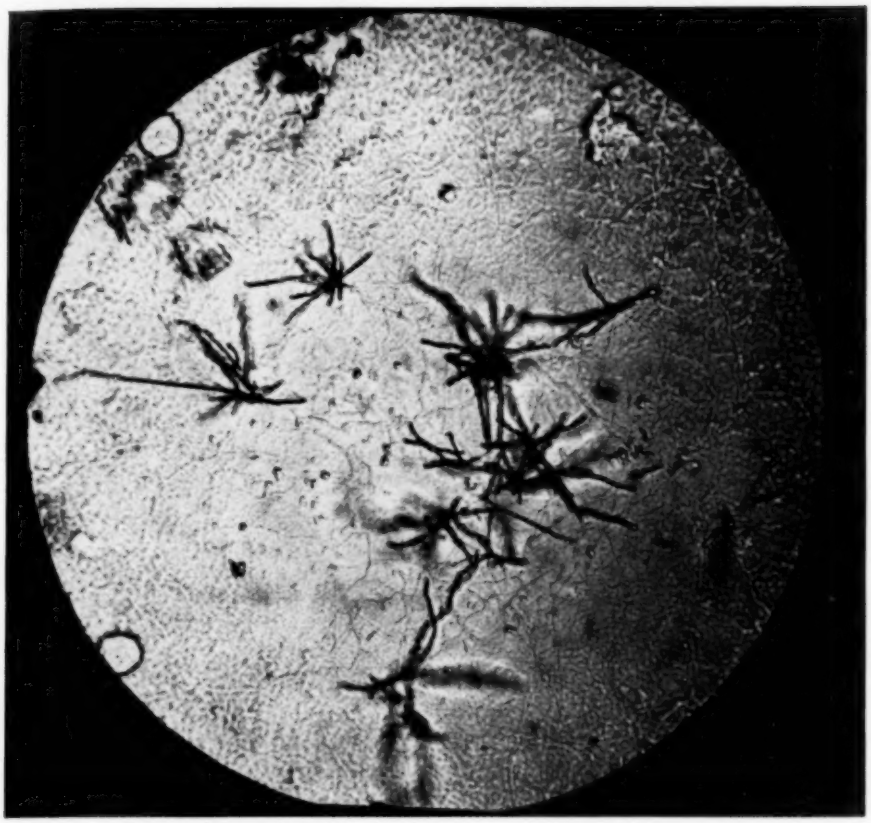


Fig. 1. *Streptomyces griseus*, streptomycin-producing strain.
Vegetative and aerial mycelium.

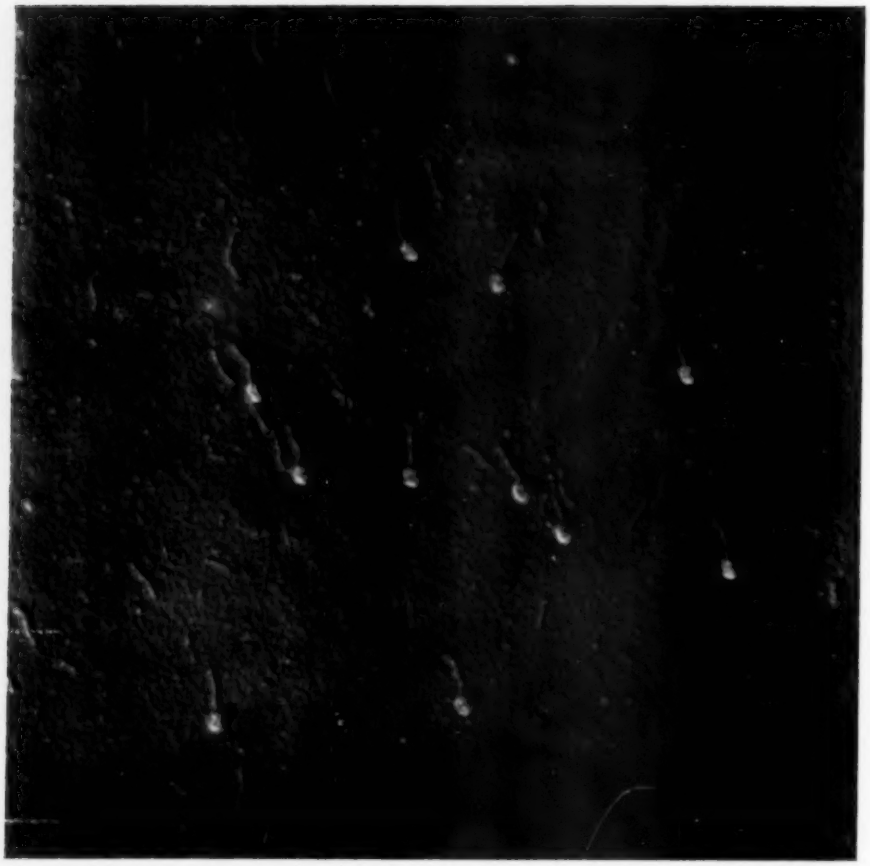


Fig. 2. Electron micrograph of actinophage, type I, of streptomycin-producing *Streptomyces griseus*, x 31,000 (courtesy of Squibb Institute for Medical Research).

The recovery of the crude streptomycin is accomplished in a series of five operations: (a) removal of the mycelium by filtration, (b) extraction of the streptomycin by adsorption on activated carbon or other adsorbents, (c) elution of the substance from the adsorbate by acid solutions, (d) neutralization of eluate and concentration by evaporation and dehydration, (e) solvent precipitation followed by filtration and drying. This results in a product containing about 25 per cent streptomycin.

Various methods have been developed for the further purification and crystallization of different forms of streptomycin. The introduction of dihydrostreptomycin, a reduced form of streptomycin, necessitated certain modifications in these procedures.

Production and Properties of Streptomycin

The production of streptomycin involves a number of problems in strain selection, life cycle of organism, and study of metabolic reactions. On continued growth in artificial media, *S. griseus* may become contaminated with a phage or virus, designated as actinophage. The growth of the organism and the production of streptomycin are both diminished, which necessitates the selection of phage-resistant strains. This is done by picking colonies from plates and testing. Those giving the highest activity are selected for the production of streptomycin. By the use of the plaque method, it is possible to measure the actual concentrations of actinophage in the culture (Fig. 2). The number of particles per 1 ml. can reach as high as 4×10^{10} to 1×10^{13}. The only way to overcome phage infestation is to develop phage-resistant strains of *S. griseus* which can be done by growing the organism in the presence of the phage. The assay of streptomycin in the culture can be carried out by means of various bacteriological and chemical methods. The former include the agar-plate diffusion or cup method, the paper disc method, the turbidimetric method, and the serial dilution method, using certain standard test organisms such as *Staphylococcus aureus, Bacillus subtilis,* or *Klebsiella pneumoniae.*[15] The chemical methods are based upon the formation of a maltol substance characteristic of the molecule, or by interaction with identifiable reagents.[16]

For standardization purposes, the measurement of concentration of streptomycin was first based upon the unit of activity, a unit being that amount of material which was just sufficient to inhibit the growth of a

[15] Waksman, S. A., and Reilly, H. C. The agar streak method for assaying antibiotic substances. *J. Industr. Engng. Chem.*, 1945, *17*, 556–558.

[16] For a comprehensive review of the various methods see *Streptomycin*, Ed. by S. A. Waksman. Baltimore, Williams and Wilkins Co., 1949.

standard strain of *Escherichia coli* in 1 mg. of medium.[17] Later, when streptomycin was isolated in a crystalline state, it was found that one unit was comparable to about 1 ml. of the pure base. It was decided, therefore, by the Food and Drug Administration, in consultation with the different manufacturers of streptomycin, to establish the potency of streptomycin on the basis of the weight of active material.

At first, the streptothricin procedure was utilized for the isolation of the crude streptomycin. When the culture broth had attained its maximum potency, usually within two to three days of incubation, it was removed from the vessels and the mycelium centrifuged or filtered off. The cell-free fluid was alkaline in reaction at the time of recovery. Active charcoal was added to adsorb the antibiotic from the culture. The adsorbate was washed with alcohol to remove some of the impurities, then with dilute acid-alcohol to bring the streptomycin into solution. The latter was neutralized and filtered, and the filtrate treated with ten volumes of ether. On treatment with acetone or by vacuum desiccation, a solid preparation was obtained. Much of the early work on the toxicity and antibacterial action of streptomycin in experimental animals was carried out with an impure product obtained in the above manner.[18–20]

Later, this method was perfected in the various laboratories working on streptomycin. The broth was first acidified to pH 2.0 and decolorized with carbon; it was then neutralized to pH 7.0, and the streptomycin adsorbed on charcoal; the adsorbate was eluted with methanolic formic acid and evaporated, or the streptomycin was precipitated with picric acid. It was then converted to the hydrochloride, chromatographed with aluminum oxide or Darco G-60, and finally converted to a crystalline compound by means of various salts, such as the helianthate.

Another purification procedure consisted in percolating an acid solution of crude streptomycin chloride in 70 to 80 per cent methanol over a sulfuric acid-washed alumina column (pH 5.0 to 6.0); an inactive fraction first appeared, which was followed by the active material. A small amount of streptomycin remained on the column; it was washed through by lowering the methanol content of the solvent. The various streptomycin fractions

[17] Waksman, S. A. Standardization of streptomycin. *Science*, 1945, *102*, 40–41.

[18] Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A. Control of gram-negative bacteria in experimental animals by streptomycin. *Science*, 1944, *100*, 103–105.

[19] Schatz, A., and Waksman, S. A. Effect of streptomycin and other antibiotic substances upon *Mycobacterium tuberculosis* and related organisms. *Proc. Soc. exp. Biol., N.Y.*, 1944, *57*, 244–248.

[20] Robinson, H. J., Smith, D. G., and Graessle, O. E. Chemotherapeutic properties of streptomycin. *Proc. Soc. exp. Biol., N.Y.*, 1944, *57*, 226–231.

obtained from the column were concentrated and lyophilized, giving white amorphous powders. The most active preparations ranged from 600 to 900 μg./mg., and accounted for about 80 per cent of the total activity. Good material was obtained only if the crude streptomycin had an activity of about 200 units per mg. or higher. Preparations of lesser purity contained substances which interfered with the purification procedures.[21,22,23] On chromatographic purifications, streptomycin concentrate gave a crystalline double salt of the trihydrochloride and calcium chloride. Other methods of isolation include adsorption procedures on amberlite resins.

Streptomycin is soluble in water and is insoluble in organic solvents, such as ether, chloroform, and acetone. The nature of the salt is of considerable importance. Streptomycin chloride is soluble in methanol, less soluble in ethanol, practically insoluble in butyl alcohol, acetic acid, and pyridine. The sulfate is only slightly soluble in methanol and practically insoluble in the other solvents.

Effects of Reaction and Various Organic Compounds

The activity of streptomycin upon bacteria is greatly influenced by the reaction of the medium and the presence of various inorganic and organic compounds. The optimum pH is 8.0; an increase in acidity results in a decrease in the bacteriostatic potency, the greatest reduction taking place between pH 6.6 and 5.9. Streptomycin is not destroyed upon contact with broth of pH 3.5 for 2.5 hours. Glucose reduces the activity of streptomycin appreciably, 2 mg. added to 10 ml. of agar being sufficient to diminish its potency by one-half. When the concentration of tryptone in the broth was increased from 0.5 to 1.0 per cent, the minimum inhibiting concentration of streptomycin against a certain test organism was also increased two or three times. The activity of streptomycin upon bacteria is not influenced to any great extent by body fluids, pus, or normal tissue juices.

In an enriched broth containing sodium glycollate, the amount of streptomycin required to inhibit growth of bacteria is about ten times greater than in the absence of this reagent. The same is true of sodium thioglycollate. Since this reagent does not cause any significant destruction of streptomycin, it was suggested that its interfering action may be due to its rôle in reducing the oxidation-reduction potential of the medium.

[21] Fried, J., and Wintersteiner, O. Crystalline reineckates of streptothricin and streptomycin. *Science*, 1945, *101*, 613–615.

[22] Kuehl, F. A., Peck, R. L., Walti, A., and Folkers, K. Streptomyces antibiotics. I. Crystalline salts of streptomycin and streptothricin. *Science*, 1945, *102*, 34–45.

[23] Carter, H. E., Clark, R. K., Jr., Dickman, S. R., Loo, Y. H., Skell, P. S., and Strong, W. A. Isolation and purification of streptomycin. *J. biol. Chem.*, 1945, *160*, 337–342.

In testing streptomycin preparations for sterility or for the purpose of determining the presence of other antibiotics, it may become necessary to inactivate the streptomycin. Hydroxylamine hydrochloride is used for this purpose. The inactivated streptomycin preparation is then added to thioglycollate containing broth. Some of the tubes are inoculated with a culture of *K. pneumoniae*. Positive growth in such tubes and negative in the controls show the sample to be sterile.

By means of cysteine, streptomycin can be differentiated from streptothricin, since the former is inactivated and not the latter.

Chemical Structure of Streptomycin

Streptomycin is a strong base, with three basic functional groups. It belongs to the glucosides, in which a diguanido-group is linked to a nitrogen-containing disaccharide-like compound. A molecular weight determination on the trihydrochloride in water gave about 800 for the free base after the necessary corrections for the chloride ion.

Upon hydrolysis, streptomycin splits into two compounds:

$C_{21}H_{39}N_7O_{12}$	+	$H_2O \rightarrow C_8H_{18}N_6O_4$	+	$C_{13}H_{23}NO_9$
Streptomycin trihydrochloride		Streptidine		Streptobiosamine

The basic nitrogen atom in the streptobiosamine is not present as a primary amino group. On further hydrolysis of the streptobiosamine with strong mineral acids, it is broken into a 6-carbon sugar and glucosamine:

$C_{13}H_{23}NO_9$	+	$H_2O \rightarrow C_6H_{10}O_5$	+	$C_7H_{15}NO_5$
Streptobiosamine		Streptose		N-methyl-l-glucosamine

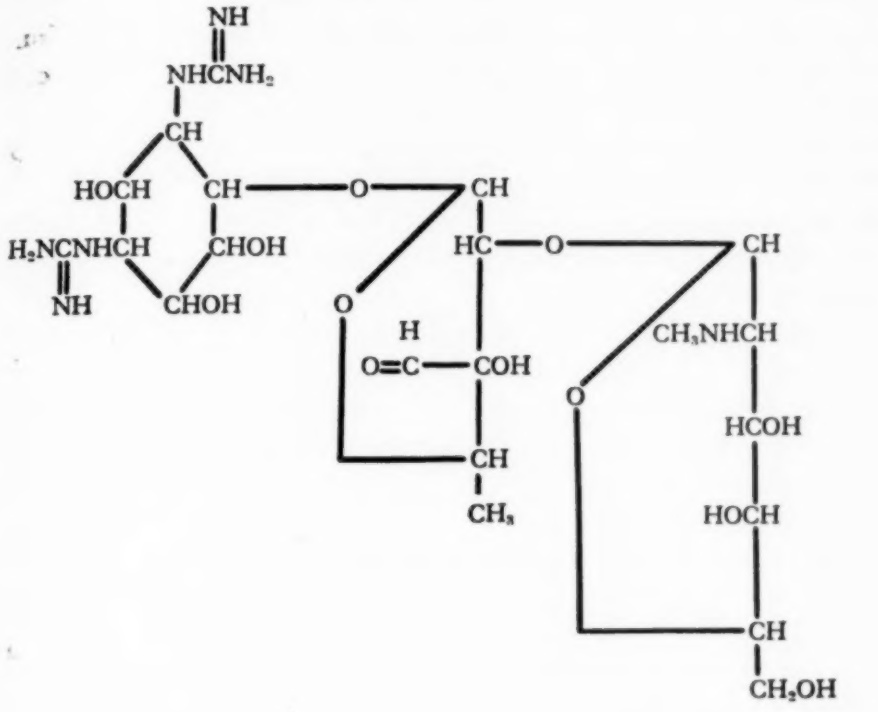
The structure of the streptomycin molecule is given below:

On catalytic hydrogenation of streptomycin, two hydrogen atoms are added to the molecule, giving dihydrostreptomycin, which is similar to streptomycin in its antibacterial and pharmacological properties, except that in many cases it exerts a less severe effect upon vestibular dysfunction.

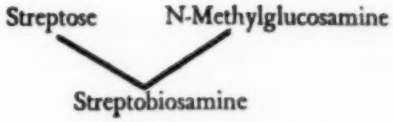
Antibacterial Properties of Streptomycin

Streptomycin is active against a great variety of bacteria found among the gram-negative, gram-positive, acid-fast, and spirochætal groups. It is not very active against anærobes, against fungi, protozoa, or viruses. Certain unknown etiological agents, such as *Granuloma inguinale,* respond readily to its effect. No absolute values can be given for the sensitivity of a given organism to streptomycin. This depends not only upon the species,

but also upon the strain, and upon the composition of the medium in which it is tested. Thus any given figure for a single organism is quite arbitrary, since such values can vary greatly.



Streptidine



Streptomycin is more active upon bacteria in young actively growing cultures than in older cultures, although this difference does not appear to be so great as in the case of penicillin. When added to a three-hour-old culture of *E. coli,* 2 μg. streptomycin reduced the numbers by 75 per cent, and 5 μg. by 95 per cent; the corresponding reductions of 24- to 48-hour-old cultures were 19 and 42 per cent. *Pasteurella tularensis* is sensitive to 0.2 to 0.4 μg./ml. streptomycin, with an initial bacterial count of two million cells per milliliter and an incubation period of seven days. A bactericidal effect is obtained by exposure to 1 μg./ml. for 30 minutes, 2 μg./ml. for 8 minutes, 4 μg./ml. for 2 minutes and 6 μg./ml. for less than 1 minute.[24]

[24] Heilman, F. R. Streptomycin in the treatment of experimental tularemia. *Proc. Mayo Clin.*, 1944, *19*, 553–558.

A study of 57 strains of *Salmonella,* comprising 15 species, gave a sensitivity range of 0.004 to 0.064 μg./ml. Five freshly isolated strains of *Klebsiella* were inhibited by 0.15 to 0.37 μg./ml., and four older cultures by 0.625 to 2.5 μg./ml. The size of the inoculum influences the bacteriostatic concentration of streptomycin. In the case of *Brucella abortus,* an inoculum of 30,000 cells required 1 μg./ml. to inhibit growth, whereas 4 μg./ml. were required for an inoculum of 30,000,000 cells. Under aerobic conditions, less streptomycin was needed to inhibit growth of various strains of *Br. abortus* and *Br. suis* than in the presence of 10 per cent CO_2. *Erysipelothrix rhusiopathiae* is also sensitive to streptomycin, although penicillin is more effective; when both antibiotics were administered in equal doses simultaneously, a synergistic action was obtained. *Hemophilus ducreyi* and other gram-negative bacteria are also highly sensitive to streptomycin. The same is true for *Phytomonas pruni* and other plant pathogens.

Streptomycin killed *Entamaeba histolytica* in concentrations of 1:2,500. It was also active against *Borrelia novyi, Leptospira icterohaemorrhagiae,* and *Treponema pallidum;* however, large doses were required for the control of the latter.

Since streptomycin is not active against viruses, it can be utilized in the isolation and purification of viruses from bacterial infections. A mixture of penicillin and streptomycin is frequently used. This combination does not interfere with virus reproduction. Bacterial contamination of influenza virus washings can thus be markedly reduced. Streptomycin frequently finds applications in virus preservation and in the growth of influenza virus in eggs in presence of bacterial contamination. It is also used in the preservation of bull semen, in the purification of cultures of *Trichomonas vaginalis* and *T. foetus,* and in the development of media for the isolation of pathogenic fungi. Because of these properties of streptomycin, it can be readily used in preventing wound infections and in surgical operations.

Among the antimicrobial properties of streptomycin, its bacteriostatic and bactericidal action upon different strains of *Mycobacterium tuberculosis* is particularly significant (Fig. 3). For the purpose of testing the sensitivity of this organism to streptomycin, the Tween medium was found to be most satisfactory (Fig. 4). A concentration of 0.05 μg./ml. inhibited growth of the human pathogen slightly, 0.2 μg./ml. produced marked inhibition of growth, and 0.4 μg./ml. complete inhibition after an incubation time of sixteen to twenty days. In other experiments, streptomycin gave complete inhibition of *M. tuberculosis* in concentrations of 0.3 μg./ml.,

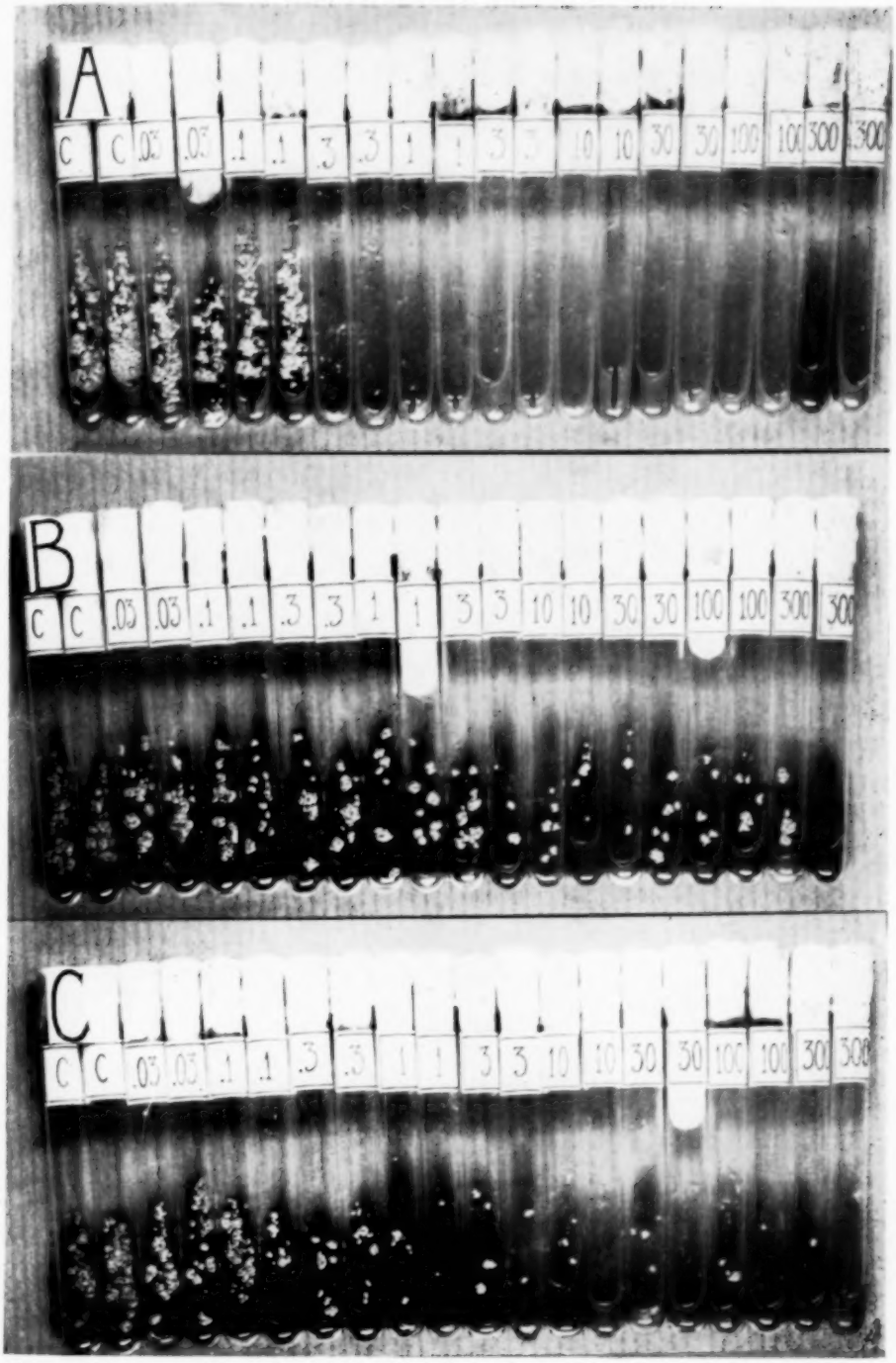


Fig. 3. First experiment in which the effect of streptomycin upon the growth of *M. tuberculosis in vitro* was demonstrated (from Schatz and Waksman).

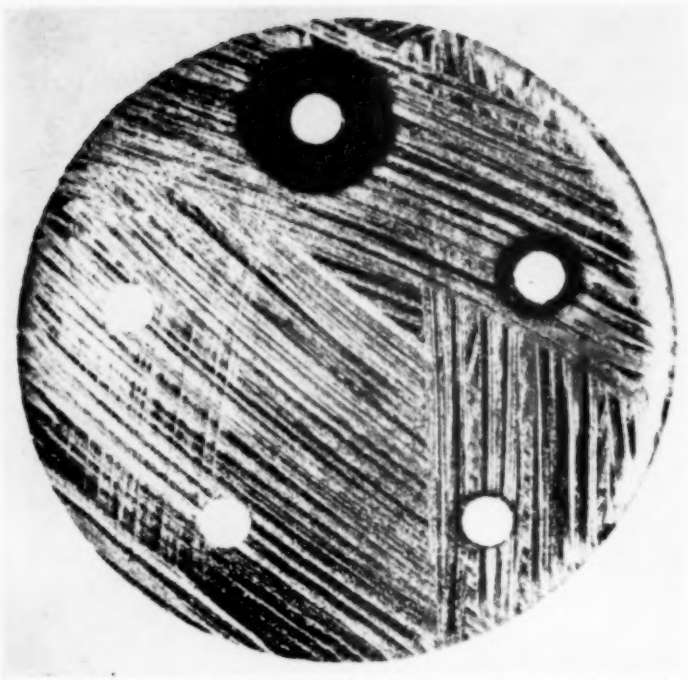


Fig. 5. Filter paper disc method for the determination of sensitivity to streptomycin (from Youmans and Fisher).

although occasional growth occurred even with 0.5 μg./ml. The bactericidal action also varied with concentration and the length of contact with streptomycin, 0.3 μg. exerting an effect in 48 hours, and 20 μg./ml. in 6 hours.

Resistance of Bacteria to Streptomycin

The problems of variation in sensitivity of bacteria to streptomycin and of increasing resistance of an organism upon prolonged contact with this antibiotic are of considerable theoretical and practical importance.

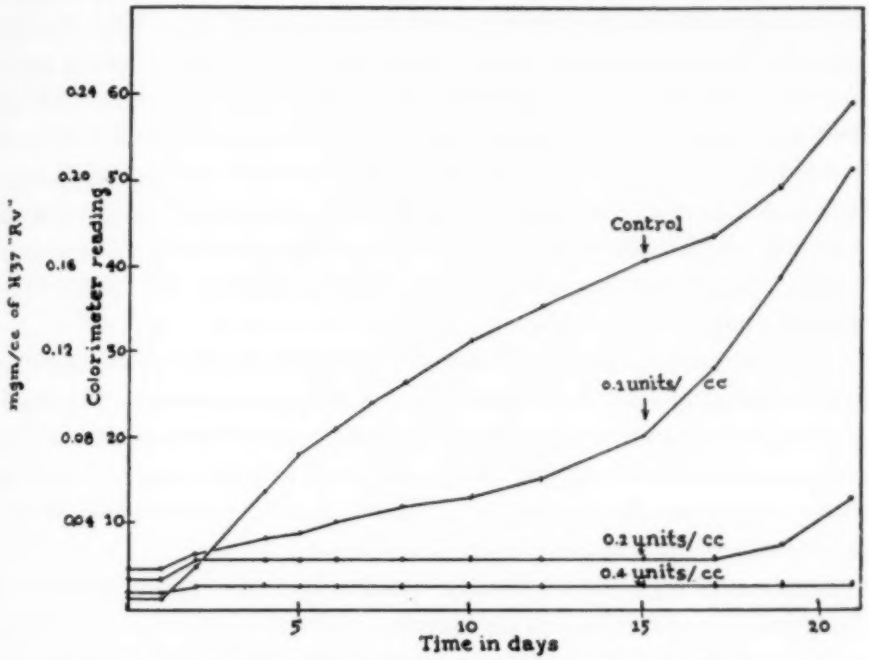


Fig. 4. Effect of streptomycin upon the growth of H37Rv in Dubos medium (from Wolinsky and Steenken).

Sensitivity to streptomycin is characteristic not only of particular species but also of given strains. Sensitivity may vary from day to day; this was believed to be partly due to variations in the number of viable organisms, in the age, and in the density of the culture. The effects of buffer of medium, the pH value, and a variety of other factors may also influence the sensitivity of an organism to streptomycin.

Freshly isolated cultures of tubercle bacilli from patients with pulmonary tuberculosis are uniformly sensitive to streptomycin, as shown in Figure 5. When a culture is exposed to streptomycin in relatively low concentrations, growth of the multiplying cells is inhibited but not of the

non-multiplying cells. Mycobacteria develop resistance to streptomycin *in vitro* at a rapid rate. This resistance persists for a considerable time and is not accompanied by any diminution in virulence. In a growing culture of tubercle bacilli, a decrease rather than an increase in the proportion of streptomycin-resistant cells took place with an increase in the age of the culture. The principal effects of streptomycin on the morphology of this organism were a loss of acid-fastness, an increase in granulation, and, in highly bacteriostatic concentrations, a shortening of the rods.

Certain strains of *S. aureus* show a natural resistance to streptomycin. This is a permanent characteristic and is not affected by passages through animals. Greater variation occurs in the degree and rate at which resistance of *S. aureus* is acquired to penicillin than to streptomycin. All the resistant strains showed some decrease in resistance upon transfer in broth free of the antibiotics. Two of the penicillin-fast strains increased in resistance to streptomycin, whereas the streptomycin-fast strains remained sensitive to penicillin. Some streptomycin-fast staphylococci showed a change in pigment production and a reduction in the rate of carbohydrate fermentation.

Maximum resistance of *S. aureus* was obtained after 12 transfers for streptomycin, 25 for streptothricin, and 32 for penicillin. A strain made resistant to streptothricin was also more resistant to streptomycin than the parent culture; however, the reverse was not true. In general, with the above exceptions, the development of resistance to one antibiotic did not produce increased resistance to other antibiotics.

Successive transfers of bacteria commonly occurring in infections of the urinary tract resulted in an increase in resistance to more than 1,000 µg./ml. of streptomycin. After 29 subsequent daily transfers on ordinary media, the bacteria lost none of their resistance. It was suggested, therefore, that streptomycin-fastness may be a major factor in the failure of streptomycin therapy in gram-negative bacillary infections. When the reservoirs of infection present physical barriers to the penetration of adequate amounts of streptomycin, as in the case of abscesses and pyelonephritis, the organisms are exposed to sublethal concentrations with a resulting increase in resistance to streptomycin. Thus in the case of infection with *Aerobacter cloacae* an increase of more than a thousand times the original resistance was obtained after 36 days' treatment.

Miller and Bohnhoff[25] made a study of six strains of gonococcus, the

[25] Miller, C. P., and Bohnhoff, M. Streptomycin resistance of gonococci and meningococci. *J. Amer. med. Ass.*, 1946, *130*, 485–488; *Science*, 1947, *105*, 620–621; *J. Bact.*, 1947, *54*, 467–482.

tolerance of which to streptomycin varied from 8 to 40 μg./ml., and of 96 strains of meningococcus, which varied in tolerance from 1 to 40 μg./ml. These organisms acquired resistance to streptomycin more readily than to penicillin, four to six daily transfers being sufficient to raise the streptomycin-resistance level to more than 75 μg./ml. The conclusion was reached that streptomycin and penicillin act on these micro-organisms in quite different ways. Gonococci and meningococci showed three other differences to the action of streptomycin and penicillin: (i) resistance to the first was acquired more rapidly than to the second; (ii) streptomycin-resistant organisms did not show any gross physical changes and abnormal microscopical appearances as did the penicillin-resistant forms; (iii) after acquiring streptomycin resistance, the meningococci retained or even enhanced their virulence, whereas the development of penicillin resistance tended to lower virulence somewhat.

Two types of variants were obtained from each of 18 strains of meningococcus, when heavy seedings were planted on a series of plates containing streptomycin in concentrations varying from 40 to 10,000 μg. per ml. One appeared in small and approximately equal numbers on all concentrations of streptomycin; it grew in the form of large yellowish colonies on streptomycin-free and streptomycin-containing media; it retained the original virulence for mice. The other strain appeared in greatest numbers on concentrations of streptomycin between 100 and 400 μg. per ml.; its colonies varied in size and color; they were small and gray on concentrations of less than 100 μg. streptomycin per ml. and larger and slightly yellowish on concentrations of 200 μg. or more per ml. The second group of variants from any strain were found to be genetically identical and the differences in their colonial appearance was determined by the concentration of streptomycin on which they grew; these variants depended on streptomycin for multiplication *in vitro* and *in vivo*. They were nonviable on media containing concentrations of less than 5 μg. per ml. and grew best on 100 to 400 μg. per ml. They were nonvirulent for mice, unless the mice received streptomycin. Both variants retained the characteristic sugar fermentations of meningococci and the type specificity of the parent strains from which they arose. Similar variants were later obtained for many other bacteria.

Streptomycin in Experimental Infections

Streptomycin proved to be highly effective in the treatment of a large number of infectious diseases brought about in experimental animals by

various gram-negative and gram-positive bacteria. Subcutaneous doses of 100 to 200 μg. of streptomycin were sufficient to protect mice against lethal infections with *S. schottmülleri* (Table 3). Oral doses of about 3 mg. were

TABLE 3
Effect of streptomycin on S. schottmülleri in mice[18]

No. of mice	Treatment	Dilution of bacterial culture used for infection	Survival of mice in hours		
			18	30	72
5	Control	10^{-5}	2	0	0
3	Control	10^{-6}	1	0	0
3	Control	10^{-7}	1	0	0
3	Control	10^{-8}	2	0	0
5	Streptomycin, 200 μg.	10^{-5}	5	5	5
5	Streptomycin, 400 μg.	10^{-5}	5	5	4

required to protect mice infected by the intraperitoneal injection of ten lethal doses. Following oral administration, streptomycin remained in the intestinal tract and gave very low blood concentrations. Urine recoveries in the animals ranged from 70 to 80 per cent when streptomycin was given parenterally and less than 10 per cent when given orally. About 5 per cent was accounted for in the bile.

The great effectiveness of streptomycin against tularemia, caused by *Pasteurella tularensis,* is shown in Table 4. Complete protection was also

TABLE 4
Effect of streptomycin on Pasteurella tularensis in mice[24]

	Mice that died Days after inoculation				No. of mice that died	Mortality rate, %
	3	4	8	23		
Treated with 1000 μg. per day (30 mice)	0	0	0	0	0	0
Treated with 500 μg. per day (12 mice)	0	0	4	1	5	42
Untreated (30 mice)	13	17	0	0	30	100

obtained in experimental animals infected with *Proteus vulgaris:* in the case of chick embryos, 1,000 μg. of streptomycin were required to arrest 18-hour

infections; to protect 50 per cent of the embryos, 250 μg. in a single dose or 150 μg. in three daily doses were required. Excellent protection was obtained by the use of 150 μg. streptomycin against fowl typhoid caused by *Shigella gallinarum,* injected into 11-day-old chick embryos.

Streptomycin had also a marked effect upon numerous other experimental infections, such as plague. *Pasteurella pestis* was inhibited by 1 μg./ml. A series of mice infected with the organism were treated with 400 micrograms streptomycin daily, beginning two days after inoculation and continuing for six days; nine of the 10 mice survived for 14 days, as compared with the survival of 4 out of 11 for the sulfadiazine treated mice and 1 out of 9 for the controls. In an experiment with guinea pigs, 48 were inoculated with the organism; a half were treated with streptomycin after clinical evidence of infection had developed; all survived, but 8 had residual buboes at necropsy; 23 controls died with plague. In another experiment, 106 guinea pigs were infected with *P. pestis* through flea transmission. After the disease was established, 15 were treated with streptomycin, of which 14 survived; 15 received sulfadiazine, of which 14 survived; 10 received both drugs and all survived; 16 received sulfapyridine and only 10 survived. Of the 50 controls three survived. Rabbits infected with the chancroid organism, *Hemophilus ducreyi,* were treated with 150 mg. of streptomycin given in three equal doses within 24 hours, treatment being started either one hour before or four hours after inoculation. The results were highly favorable.

Infections due to various gram-positive bacteria were found to be somewhat more resistant to the action of streptomycin. Nevertheless, experimental infections caused by *Diplococcus pneumoniae* and *S. aureus* were readily controlled when adequate doses were employed.

Of particular importance were the experiments of Feldman and Hinshaw[26,27] on the effectiveness of streptomycin in experimental tuberculosis in guinea pigs (Table 5). On the basis of an arbitrarily established index of infection, microscopically determined, 100 represented the maximum possible amount of tuberculosis. The control animals, sacrificed after 61 days, exhibited an index of 67 as contrasted to 5.8 for those which had received streptomycin. In another experiment, the corresponding values were 81.9 for the untreated and 2.8 for the treated animals. The daily administration of streptomycin per pig varied from 1,387 to 6,000 μg.

[26] Feldman, W. H., and Hinshaw, H. C. Effects of streptomycin on experimental tuberculosis in guinea pigs: a preliminary report. *Proc. Mayo Clin.,* 1944, *19,* 593–599; 1945, *20,* 313–318.

[27] Feldman, W. H., Hinshaw, H. C., and Mann, F. C. Streptomycin in experimental tuberculosis. *Amer. Rev. Tuberc.,* 1945, *52,* 269–298.

TABLE 5

Effect of streptomycin upon the development of human tuberculosis in guinea pigs[26]

Experiment No.	Number of animals	Duration of infection, days	Duration of treatment, days	Index of Infection
1 Controls	8	43–60	0	81.9
1 Treated	4	54	39–54	2.8
2 Controls	9	61	0	67.0
2 Treated	9	61	47–61	5.8

Two different strains of the human tubercle bacillus were equally sensitive to streptomycin *in vivo* (Fig. 6). *M. tuberculosis* was recovered from the spleens of only one of the guinea pigs treated with streptomycin, the animal having received 1,387 μg. streptomycin daily for 54 days. The period of treatment was very important (Fig. 7).

Untreated control animals gave the usual advancing morbid processes in the spleen, lungs, and liver. Microscopical examination of these organisms from each of the streptomycin-treated animals showed a totally different situation. No lesions were found in the lungs of any of the guinea pigs, whereas the livers of only two were involved. The spleens were also largely free from infection. The antibiotic for the most part prevented the development of tuberculosis in guinea pigs when treatment was started on the same day that the animals were inoculated. It also showed a suppressive effect in those animals that had been inoculated two weeks prior to the beginning of treatment.

The conclusion was reached that streptomycin is the most effective *in vivo* tuberculochemotherapeutic agent which has so far been studied. The relatively low toxicity of streptomycin for guinea pigs and its high efficacy in resolving and suppressing what would otherwise be lethal tuberculosis in guinea pigs establish streptomycin as a drug worthy of serious consideration for the treatment of tuberculosis.

TOXICITY OF STREPTOMYCIN

To produce toxic manifestations of streptomycin in animals, it is necessary to administer either extraordinarily large amounts of the pure

material or smaller amounts of certain impure preparations. No correlation was obtained between the acute toxicity of different lots of experimental animals and clinical tolerance by human beings. The behavior of

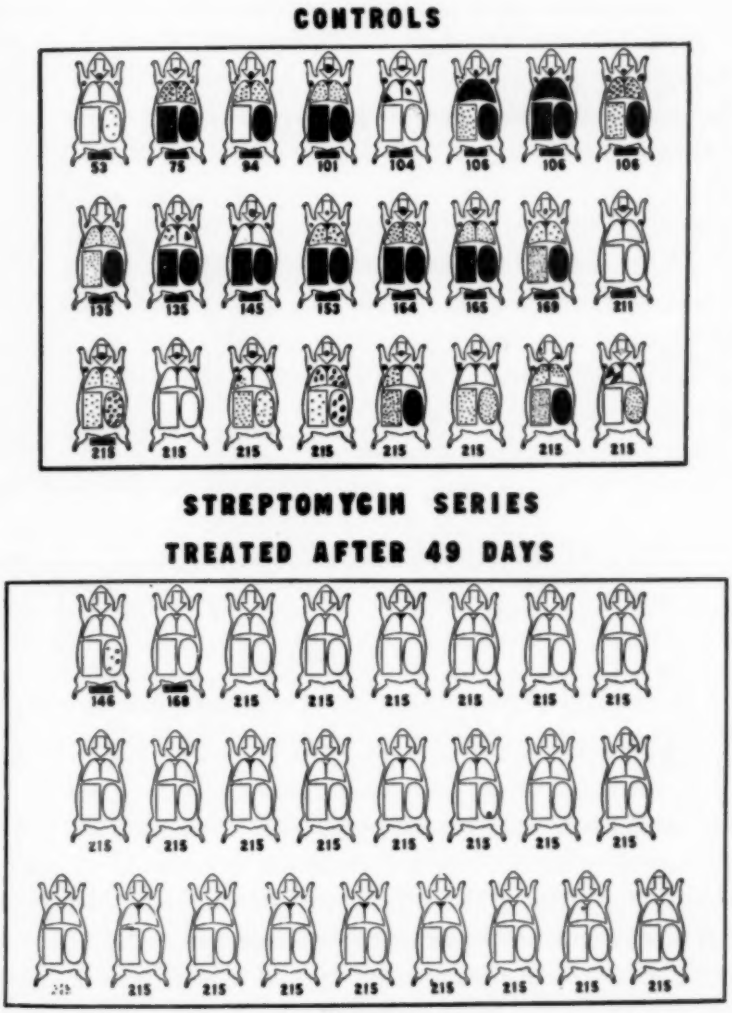


Fig. 6. First experiment in which the effect of streptomycin on experimental tuberculosis in guinea pigs has been established (from Feldman, Mann, and Hinshaw).

the material in man cannot, therefore, be foretold if antibacterial potency alone is assumed to be the criterion of purity; highly concentrated material has on occasion been unsatisfactory clinically, whereas considerably less active preparations have often produced no undesirable effects.

Among the various toxic reactions resulting from the administration of streptomycin, the otic complications received particular attention. In one experiment, 81 patients treated with streptomycin were examined for

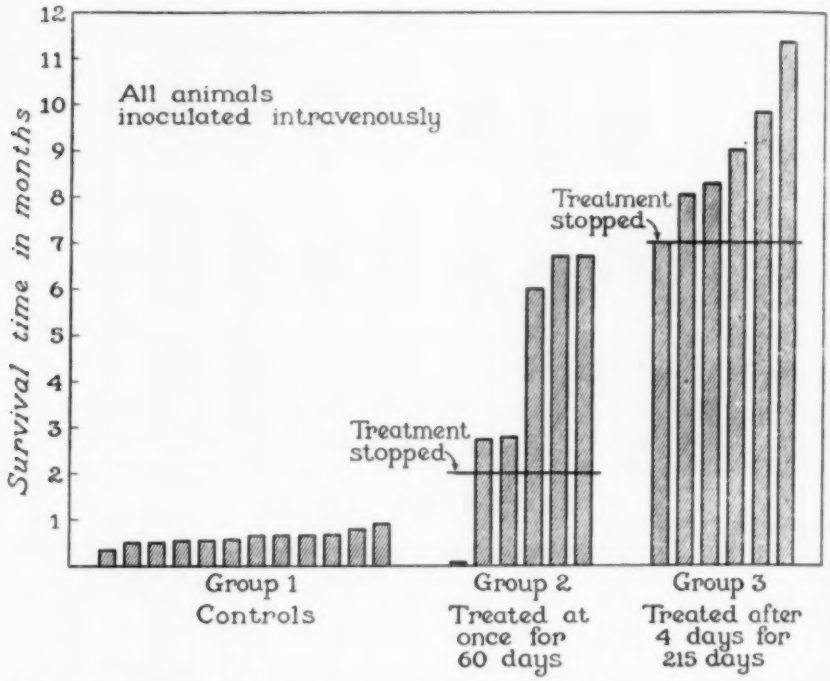


Fig. 7. Relative survival time of three groups of guinea pigs inoculated intravenously with 1 mg., moist weight, of tubercle bacilli, strain H37Rv (from Feldman and Karlson).

evidence of aural toxicity. Two months after the cessation of therapy, three of these patients showed an absence of vestibular response. During the third month after therapy, one of these three showed increasing recovery of response. All three complained spontaneously of dizziness during treatment, which decreased steadily. One patient showed a high tone loss, but the audiogram was normal in these three patients. Another patient showed a severe loss of hearing two weeks after treatment. Two weeks later he had shown decided improvement in hearing. He had a normal vestibular response throughout. It was not certain that the low tone and high tone losses were due to streptomycin. The introduction of dihydrostreptomycin and the use of smaller doses tended to reduce the toxic manifestations of the drug. Although some patients developed drug fever and some had a rash, the symptoms were cleared when administration was discontinued.

Administration of Streptomycin

Streptomycin is administered parenterally (subcutaneous, intramuscular), intrathecally, or orally, depending upon the nature of the infection. The dosage varies from one patient to another depending on the type and severity of infection. Since acquired resistance occurs rapidly in some patients with infections due to susceptible organism, maximum doses should be used from the beginning. For intermittent intramuscular injections, 1 or 2 ml. of distilled water or physiological saline solution containing 125 mg./ml. of streptomycin are given every three or four hours. Occasionally satisfactory clinical results are obtained by administering every six hours, the total daily dose being 1 to 2 grams. For continuous intramuscular administration, the daily dose of 1 to 2 gm. is dissolved in 500 to 1,000 ml. of physiological saline solution, the rate of flow being properly regulated. Streptomycin is rapidly excreted in the urine within 24 hours after injection, depending on concentration and manner of administrations. Doses of 500 mg. given every six hours maintained a serum level adequate to combat bacteria whose growth was inhibited by concentrations of 5 to 7.5 μg. streptomycin/ml. of culture medium. Little streptomycin diffuses from the blood into the cerebrospinal fluid and only minimal amounts are found in the blood following inhalation or oral administration. Following parenteral injection, streptomycin passes into the pleural fluid, the peritoneum, ocular fluid, and the bile. It also passes through the placenta into the fetal circulation and amniotic fluid. It has been found in the umbilical cord blood and amniotic fluid within ten minutes following intravenous injection into the maternal circulation. The concentration in the cord blood is less than half that in the maternal blood. When high concentrations are desired in the pleural cavity, it is well to inject material directly into it.

Relatively little transfer of streptomycin occurs between the blood and the lumen of the gastro-intestinal tract in either direction. Following oral administration, levels as high as 9 mg. per gm. were found in the feces. Due to the poor transfer of streptomycin across the walls of the alimentary tract, it was suggested to use it both orally and parenterally in the treatment of infections such as those in which the pathogenic organisms are found both in the gastro-intestinal tract and in the blood stream. Following oral administration of 420 mg. of streptomycin to dogs, for example, none was detected in the plasma, although up to 3.9 per cent was recovered in the urine. Following a single oral dose of 500 mg. to humans, none was

absorbed from the gastro-intestinal tract; however, after doses of 1 and 2 gm., traces appeared both in the blood stream and in the urine.

Streptomycin has proved to be very effective as an aerosol; nebulization with streptomycin (500 mg. in 20 ml. physiological saline solution each day) led to rapid disappearance of the gram-negative bacteria from the pulmonary secretions, accompanied by an immediate reduction in the volume of sputum; the remaining sputum lost its purulent character.

DISEASES RESPONDING TO STREPTOMYCIN

A variety of human and animal diseases caused by various bacteria respond readily to streptomycin treatment. This was brought out in the first comprehensive study of a group of diseases which could be considered either to be definitely controlled by streptomycin treatment or to give promise that they would respond favorably. These included tularemia, urinary tract infections, especially those resistant to sulfa drugs and to penicillin, *Klebsiella* and *Hemophilus* infections, bacteremia due to penicillin-resistant organisms, various forms of meningitis, and whooping cough. Streptomycin was also helpful in a variety of other diseases, such as leprosy, typhoid fever, brucellosis, certain forms of tuberculosis, and probably also in bacillary dysentery, cholera, and bubonic plague.

The Committee on Chemotherapy, originally organized by the Committee on Medical Research of the Office of Scientific Research and Development, undertook the supervision and co-ordination of the first large-scale series of investigations on the use of streptomycin in the treatment of bacterial infections.[28]

The first thousand cases were thus evaluated. In 409 cases of urinary tract infections, the general recovery rate was 42 per cent. Of 100 cases of *H. influenzae* meningitis, recovery was obtained in 79 per cent; in 17 of the fatal cases treatment was given too late to be effective. In 14 cases of meningitis caused by other gram-negative organisms, 4 died; early streptomycin treatment was recommended in all such cases. In 91 bacteremia cases, 49 recovered, 12 improved, 26 died; in this group as well, the fatal cases were treated too late. Striking results were obtained in tularemia (63 recoveries out of 67 cases), and in otitis media (7 immediate recoveries out of 8 cases). In 44 cases of pulmonary infections, recovery or improvement

[28] Keefer, C. S., Blake, F. G., Lockwood, J. S., Long, P. H., Marshall, E. K., Jr., and Wood, W. B., Jr. Streptomycin in the treatment of infections. A report of one thousand cases. *J. Amer. med. Ass.*, 1946, *132*, 4–10, 70–77.

was obtained in 61 per cent. Streptomycin was extremely effective against acute Friedländer bacillus infections. No dramatic effects were observed in the 45 cases of acute brucellosis. In 51 cases of typhoid, streptomycin accelerated return of temperature to normal, if administration was started early. Of 26 cases with *Salmonella* infection, 12 recovered, 8 died. Here again, maximum, early, and continuous streptomycin treatment was recommended. Encouraging results were obtained in peritonitis, where 39 recoveries were obtained in 53 cases.

Streptomycin in Tuberculosis

In the report of the above Committee, evaluation of streptomycin in tuberculosis was based on 75 cases. The results observed in miliary, meningeal, laryngeal, skin, and renal tuberculosis, were considered as highly suggestive, namely, that streptomycin exerts a bacteriostatic effect on the growth of the tubercle bacilli in man. The same was believed to hold true for exudative pulmonary tuberculosis. It was recommended that a minimum period of treatment should be three to six months with daily doses of 1.5 to 3.0 gm.

Hinshaw, Feldman, and Pfuetze[29] reported the results of treatment of 100 tuberculosis cases. They came to rather optimistic conclusions, namely, that streptomycin is an antibacterial agent which possesses the unique ability to inhibit the growth of *M. tuberculosis* both experimentally and clinically. It was recommended that treatment should not be undertaken unless adequate amounts of streptomycin were available to complete a course of at least two to four months' duration, the total requirements being about 360 gm. It was also emphasized that toxic manifestations should be anticipated in a majority of patients. Disturbance of equilibrium was believed to be especially likely to occur, often persisting for several weeks after the discontinuation of streptomycin therapy.

Further studies brought out the fact that various forms of tuberculosis respond promptly to treatment with streptomycin. These included ulcerating tuberculous lesions of the larynx, hypopharynx, and the tracheobronchial tree that received combined intramuscular and aerosol treatment; some types of extrapulmonary tuberculosis have also responded well, especially chronic, long-standing draining sinus tracts, which usually close within a few weeks, but remain closed only if treatment is continued for

[29] Hinshaw, H. C., Feldman, W. H., and Pfuetze, K. H. Streptomycin in treatment of clinical tuberculosis. *Amer. Rev. Tuberc.*, 1946, *54*, 191–203.

several weeks after superficial healing. Temporary and palliative effects have been observed in renal tuberculosis and tuberculous cystitis, although actual healing of renal lesions has been observed rarely, possibly because the kidney lacks the power to heal tuberculosis readily. Encouraging results have also been obtained in the case of tuberculosis of bones and joints. Some patients with early tuberculous meningitis responded promptly to the adequate intramuscular and intrathecal administration of streptomycin, but frequently these gains were not permanently sustained; however, temporary clinical remission was frequently realized after streptomycin treatment of early but acute and severe tuberculous meningitis; consciousness may be regained, sometimes within a few days, fever may decline within a few weeks, the patient appearing normal for several months; subsequent exacerbations of the diseases are likely to occur, and not likely to respond to treatment. Three patients with proven tuberculous meningitis were treated; the arrest of the disease continued for several months after discontinuation of a six-month course of intensive treatment.

Disseminated hematogenous tuberculosis of the miliary type did not respond previously to treatment, spontaneous recoveries being extremely rare. By the use of streptomycin it was possible to bring about a complete clinical and roentgenological remission in some treated patients; unfortunately, a high recurrence took place. Tuberculosis of the alimentary tract and tuberculous peritonitis have showed striking symptomatic improvement.

In describing the effectiveness of streptomycin in tuberculosis, Hinshaw and Feldman emphasized that tuberculosis, by virtue of its usual chronicity, produces destructive changes in the tissues of the host. Healing or repair of these tissues is exceedingly slow. Furthermore, in extensive tuberculosis of the lungs the destructive changes offer serious handicaps to healing. When such handicaps exist, a corrective mechanical type of treatment, such as the conventional surgical collapse procedures, is used rather than treatment with streptomycin. The physician, therefore, could not hope for any alternative chemical remedy when surgery is indicated. It was suggested that rest therapy, usually in the planned environment of a sanatorium, would probably remain the fundamental remedy for tuberculosis. The rôle of streptomycin was said to consist in blocking the paths for extension of the disease while the healing forces are operating.

A typical series of results is presented in Table 6. On the basis of these and numerous other clinical evidence, Walker, Hinshaw, and Barnwall[80] felt justified in coming to the following conclusions:

The introduction of streptomycin into clinical medicine has been peculiarly exciting to phthisiologists. Not only have they never had an 'antibiotic' which was helpful in the treatment of tuberculosis in man, they have never had a proved effective drug of any sort. So far as chemotherapy is concerned, not only was their situation comparable to that of the syphilologist prior to the discovery of penicillin, it was worse than his position before the appearance of Ehrlich and arsenic. Phthisiologists have been dependent on rest in bed, which finds its climax in the 'lung immobilizer' and, more recently, on collapse therapy, which has become most definitive in the form of excisional surgery.

Chemotherapy is a radical departure from tradition in tuberculosis, and on this account it might have been anticipated that a tuberculostatic drug would be slowly, perhaps inaccurately, evaluated. In the case of streptomycin, this would appear not to have occurred. Within 3 years after publication of the first clinical paper describing its use, it has been possible to write a chapter of this sort—tentative and incomplete in many respects, but unlikely to be regarded as complete nonsense within the next year or so. This relatively rapid evaluation of a new drug—rapid for tuberculosis if not for pneumococcic pneumonia—is partly the result of experience gained from the investigation of other drugs in other diseases. It is partly a result of the employment, here and in England, of cooperative methods of investigation which have yielded data, reasonably uniform, rapidly, and in large amount; methods which were used, perhaps for the first time in the beautifully organized study on penicillin and syphilis which was initiated during the recent war.

TABLE 6

Changes occurring in 382 patients with 687 draining cutaneous sinuses and fistulae treated with streptomycin[30]

Period of treatment—120 days

Amounts used, gm per day	1.8–2.0	1.0	0.5	0.2
Patients	126	229	15	12
Sinuses and fistulae	257	403	15	12
Observations at end of treatment	Incidence, per cent			
Healed (average 8 weeks)	87.3	69.6	60.0	83.3
Improved	7.8	23.5	40.0	16.7
Unchanged	4.6	6.9	0.0	0.0
Worse	0.3	0.0	0.0	0.0
Recurrence after healing	2.0	2.5	0.0	0.0

MANUFACTURE OF STREPTOMYCIN

Manufacture of streptomycin was begun in 1944, first by Merck & Co. and later, in 1945, by other American manufacturers. At present, seven

[30] Walker, A. M., Hinshaw, H. C., and Barnwell, J. B. Streptomycin in the treatment of tuberculosis in man. In: *Streptomycin*. Ed. by S. A. Waksman. Baltimore, Williams and Wilkins Co., 1949, pp. 291–319.

American companies are producing streptomycin, and about fifteen companies in foreign countries. The production and prices are given in the following summary:

Year	Production* (1,000 gm.)	Exports (1,000 gm.)	Price (per gr.)**
1945	c. 300	—	—
1946	1,175	45	4.69
1947	9,676	c. 3,450	3.16
1948	37,709	c. 26,500	1.57
1949	83,699	63,412	0.88
1950	92,446	66,419	0.60
1951 (1st qt.)	31,140	16,523	0.60

* Streptomycin and dihydrostreptomycin.
** Price to physicians originally $25 per gram.

It may be of interest to note that in certain European countries there has been, during the last five years, a large black market on streptomycin. Although the official export figures for streptomycin in 1949 exceed the sum of forty million dollars, one may safely add a few more millions to account for the numerous parcels sent by Americans to their friends and relatives in Europe and those quantities which were "bootlegged" out of the country.

SUMMARY

A strain of *Streptomyces griseus* capable of producing a new antibiotic was first isolated in August in 1943. This antibiotic was designated as streptomycin. It was very similar to streptothricin, which was isolated in the same laboratory two years previously. Since methods for the production and chemical purification of streptomycin, as well as the determination of its potential activity against various bacteria, were worked out previously for streptothricin, the progress made in the isolation and recognition of streptomycin was very rapid. The crude material was submitted immediately to animal experimentation, where positive results were quickly obtained. Before the end of 1944, the effect of streptomycin in experimental tuberculosis was established. Its value in the treatment of numerous infectious diseases of man, which had not previously lent themselves to therapy, was soon recognized. These included numerous diseases caused by gram-negative bacteria and by the tuberculosis organism.

Before another year had passed, namely, in 1945, the importance of streptomycin as a chemotherapeutic agent in the treatment of numerous

infections was well established. Although some of the newer antibiotics have tended to replace streptomycin or to supplement it in the treatment of diseases caused by gram-negative bacteria, such as urinary tract infections, brucellosis, and tularemia, streptomycin has remained by far the most important therapeutic agent for the treatment of tuberculosis.[31]

The discovery of streptomycin as a product of a rather obscure group of micro-organisms, namely, the actinomycetes, has pointed a way to the study of these organisms as potential producers of other chemotherapeutic substances. It may be said that since the rediscovery of penicillin in 1940, the most important antibiotics that have found application as chemotherapeutic agents are produced by actinomycetes.[32]

[31] Cairns, H., Smith, H. V., and Vollum, R. L. Tuberculous meningitis. *J. Amer. med. Ass.*, 1950, *144*, 92–96.

[32] A complete summary of the history and literature on streptomycin is found in the following books:

Waksman, S. A., *Microbial antagonisms and antibiotic substances*. 2d ed. New York, The Commonwealth Fund, 1947.

Waksman, S. A., Ed., *Streptomycin*. Baltimore, Williams and Wilkins Co., 1949.

Fanconi, G., and Loffler, W. *Streptomycin and Tuberkulose*. Basel, Benno Schwabe Co., 1948.

Choremis, K. V. *The pharmacology of streptomycin in the therapy of tuberculosis in children*. Athens, I. A. Aleiropoloi, 1948.

Bustinza, F. *De Koch a Waksman*. Madrid, Espasa-Calpe, S. A., 1948.

Morin, M., Nehlil, J., and Pichon, R. *La streptomycine*. Paris, Masson et Cie (1949).

Zorini, A. O., and Cattaneo, C. *Streptomicina e Tubercolosi*. Roma, Sansoni ediz. scientifiche, 1950.

Pharmacology of Antibiotics

HENRY WELCH*

THE impetus given to the search for new antibiotics after the discovery of tyrothricin by Dubos in 1939 and the development of penicillin during World War II has resulted in the reporting of literally hundreds of new and untried antimicrobial substances. There has been compiled recently a dictionary of these substances in which are listed some thousands of antibiotics and, because of the large number of screening programs in progress, their number is increasing. It is significant, however, that of these thousands of antimicrobial substances less than ten have been found to be useful in the treatment of human or animal diseases.

The pharmacological study of a new antibiotic is usually not a simple one, particularly if the agent is impure. It will be remembered that both penicillin and streptomycin when first used clinically were relatively crude drugs, much more so in the case of penicillin than streptomycin. As a result, some of the toxicity first attributed to penicillin, such as irritation on injection and damage to nerve tissue, could not be immediately assigned to the pure crystalline substance per se. The purification of penicillin revealed that intramuscular irritation was related to purity while toxicity for nerve tissue was inherent in the drug itself. At the present time it is difficult, if not impossible, to determine whether the nephrotoxicity of bacitracin and neomycin is due to impurities or whether it is a quality inherent in the drugs themselves, though it appears that the latter is the case. The "broad spectrum" antibiotics—aureomycin, chloramphenicol, and terramycin—are crystalline drugs which contain few if any impurities. Thus any toxicity demonstrated for them can be attributed to the drugs themselves. It is perhaps unfortunate that, because of the need, it was necessary to use penicillin and streptomycin prior to their purification to the crystalline state. However, the progress made in the antibiotics field during the past ten years has been such that it is relatively unlikely that in the future we shall be required to determine the pharmacology of or to use many new antibiotics which have not been purified. In the following discussion of the pharmacology of antibiotics, only those chemotherapeutic substances which are currently in use will be discussed, keeping in mind that the pharmacology of some was determined on the impure drug, while others were studied in the pure state.

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Tyrothricin

The tyrothricin available commercially consists of an impure defatted mixture containing approximately 20 per cent gramicidin and 80 per cent tyrocidine. Tyrothricin, gramicidin, and tyrocidine are highly active poisons. Both tyrothricin and tyrocidine hemolyze erythrocytes. Gramacidin has been shown to be hemolytic as well, but its action is delayed and the lytic activity of tyrothricin is usually ascribed to its tyrocidine content. The lysis caused by tyrocidine is prevented by the presence of serum. Gramicidin is more toxic than tyrocidine for cells in general, and the cytotoxicity of tyrothricin is largely due to its gramicidin content.

Injection of small amounts of these drugs in animals by the intravenous, intramuscular, intraperitoneal, or subcutaneous routes invariably causes death in a relatively short time. Death is apparently due to respiratory failure since the heart may continue to beat for some time after breathing ceases. Animals dying after lethal doses of these drugs show nonspecific degenerative changes. Acute congestion of the lungs and abdominal viscera, petechial hemorrhages of lungs, kidney, and myocardium, and diffuse hemorrhages of the spleen have been reported. Histopathological examination usually shows fatty degeneration of the liver with necrosis, cloudy swelling of the tubular epithelium of the kidney, and hemorrhage of the glomeruli. Orally tyrocidine and gramicidin are of relatively low toxicity.

In man, no appreciable toxicity of tyrothricin has been reported following local application. During the past two years, increased commercial availability of tyrothricin preparations has emphasized the need for cautious use of this drug. Following its use as nose drops with or without a vasoconstrictor, both parosmia and anosmia may occur. More serious, however, is the possibility of chemical meningitis following the use of solutions of tyrothricin for irrigation of cavities near the subarachnoid space. Fatal chemical meningitis has been reported after such use of tyrothricin solutions. Following injection of 1 cc. of 1:1,000 tyrothricin in alcohol into the cisterna magna of dogs, death occurred immediately or within a few hours in 50 per cent of the animals so treated. The pathological process in the surviving animals was consistent with acute purulent chemical meningitis. The final picture in those animals which survived revealed gross and microscopical thickening of the leptomeninges with or without hydrocephalus. One animal was observed to have a fully developed hydrocephalus involving all ventricles.

The observations in patients, together with the reproduction of the

pathological processes in animals, indicate that tyrothricin solutions should not be used for the irrigation of sinus cavities in close proximity to the subarachnoid spaces and emphasize again the inherent toxicity of this drug.

PENICILLIN

Penicillin is by far the most widely employed of the antibiotics now available to the general practitioner and in many of the bacterial infections it remains the drug of choice. The toxicity of penicillin was shown early to be extremely low and, in general, the impressions gained at that time have been borne out in the extensive use of this drug. The original studies showed that even with relatively crude preparations there was little to indicate contraindication to its use in man. Further studies substantially confirmed the fact that crude amorphous penicillin had little, if any, true toxicity, although an occasional sensitization occurred with this material. As early as 1944, at which time only amorphous material was available, it was demonstrated that insofar as the calcium and sodium salts of penicillin were concerned, its acute toxicity appeared to be associated with the cation rather than with penicillin per se. Later studies of the sodium, lithium, ammonium, strontium, calcium, magnesium, and potassium salts showed conclusively that the toxicity of these penicillin salts was primarily associated with the cation. These studies were carried out with penicillin salts that were not pure although their potencies were on the order of 1,000 units per mg. The apparent toxicity of penicillin decreased as the purity of commercial preparations improved. Therefore it is important in discussing the pharmacology of penicillin to give consideration to the fact that all of the reports concerning toxicity made prior to the advent of crystalline penicillin G were based on amorphous penicillin containing impurities, which may have been responsible for certain of the toxic effects ascribed to penicillin itself. It should be emphasized that the penicillin commercially available today is largely crystalline penicillin G. Amorphous penicillin is no longer produced. The presently manufactured penicillin approaches the theoretical potency—1667 units of activity per mg. (60 mg. per 100,000 units).

One of the toxic effects observed with amorphous penicillin, an abrupt rise in temperature following injection, was due, without doubt, to pyrogenic impurities. The presence of these pyrogenic substances in early commercial lots of penicillin resulted in specifications by the Armed Forces, who procured all of this drug during World War II, which required animal tests to demonstrate the absence of pyrogenic substances. Likewise,

safety tests in mice were included to eliminate the possibility that chance contamination with solvents and other materials might render the product harmful.

The doses utilized, even in early clinical trials of penicillin, were relatively high and were a direct indication of the extremely low toxicity of this drug. At the present time, doses as high as 100,000,000 units daily of crystalline sodium or potassium penicillin G are given without untoward effects. There is little or no pain following intramuscular injection of crystalline penicillin. However, early lots of amorphous penicillin of low purity were quite painful on injection. As a matter of fact, there was a significant correlation between the purity (potency) of commercial sodium penicillin and irritation following intramuscular injection. With an increase in potency in units per mg., there was a corresponding decrease in the pain produced.

The early indications were that penicillin produced no serious toxic effect on the nervous system. However, there is no question now, that even with the pure crystalline drug, nerve tissue is vulnerable to its action. Convulsions may follow intraventricular administration of penicillin in man, and can be induced in animals by direct application of penicillin to the cerebral cortex. Neurological complications in patients with pneumococcic meningitis have been noted after intrathecal administration of penicillin. In these cases, neurological symptoms were manifest between the tenth and twenty-third day after the institution of therapy. Both motor and sensory disturbances were observed although recovery was eventually complete. Peripheral neuritis has also been reported as a complication of intrathecal penicillin therapy. Localized peripheral neuritis with motor and sensory disturbances has been observed following intramuscular administration of penicillin. Recovery from the neuritis occurs in most cases. Symptoms following intrathecal administration of penicillin, such as listlessness, headache, nausea, vomiting, respiratory difficulty, cyanosis, fall in blood pressure, thready pulse, and muscular twitching, are apparently reduced or eliminated when the dosage is diminished. This is particularly true if barbiturates are used concomitantly with reduction in the dose. When 5,000 units of crystalline penicillin are applied to the occipital cortex in human beings, no clinical or electroencephalographic abnormalities occur, whereas 20,000 units of the same material produce definite encephalographic changes. Indications are that the intrathecal reactions are related to the size of the dose rather than to the concentration, acidity, or alkalinity of the solution used. The irritating effect of penicillin on the central nervous

system can be diminished directly in proportion to the destruction of the antibacterial properties of the drug.

Toxic reactions to oral therapy with penicillin have been reported on numerous occasions. Black tongue or glossitis may occur in patients treated with troches or with troches and sprays. Stomatitis following the use of penicillin as troches or by inhalation has been reported. There are other side effects resulting from penicillin therapy which may be classified as rather indefinite reactions. These include gastro-intestinal disturbances, muscle cramp, flushing of the skin, and headache. These reactions are observed not infrequently and some have been described as being allergic in nature. The symptoms are difficult to explain but rarely, if ever, are they sufficiently severe to necessitate withdrawal of the drug. One of the serious reactions to penicillin in man is the Herxheimer reaction in syphilis. This "therapeutic shock" presumably represents a reactivation of the syphilitic process which sometimes follows the use of any potent antisyphilitic drug. The Herxheimer reaction has followed the use of penicillin in both early and late syphilis and alarming manifestations may occur in cases of cardiovascular and neuro-syphilis. Deaths have occurred presumably following Herxheimer reactions during the use of penicillin therapy in syphilis. Fortunately, this type occurs infrequently.

Of more importance because of their possible universal occurrence are the allergic manifestations which may be observed following use of penicillin. The early belief that allergic reactions were connected exclusively with the impurities in penicillin has been thoroughly discredited by the mass of data to the contrary. Undoubtedly the impurities in amorphous penicillin increased the possibility of sensitization, but the pure crystalline drug itself is quite capable of causing an allergic reaction in man. Sensitization with crystalline penicillin approximating the theoretical potency of 1,667 units per mg. may occur in individuals who have never had previous contact with the drug. About 5 per cent of individuals exhibit a positive reaction of the tuberculin type to penicillin, despite the fact that they have not had previous contact with the drug. In addition to those individuals who are sensitive on primary contact with penicillin, there is a group of probably 10 per cent of the population that may become sensitive following brief or prolonged treatment. Certain individuals become sensitive following a few injections and others require many.

Probably the most common reaction to penicillin is urticaria. This may vary from a single or few transient erythematous wheals to the diffuse massive plaques of angioneurotic edema which may be localized on the

hands, lips, eyelids, vulva, or larynx. Epidermal hypersensitivity to penicillin is more likely to occur following topical application. The number of cases of contact dermatitis following topical application of penicillin makes one question the value of this drug for local therapy. In addition to contact dermatitis, an Arthus type of reaction and a condition resembling serum sickness are encountered. Reactions are seen most frequently after topical application of the drug but similar reactions have occurred following parenteral administration after initial topical use, and after parenteral or oral administration only. In some instances, reactions occur after initial exposure and in others after subsequent administration. In any case, the increase in frequency of reactions observed following penicillin therapy indicates that local application of the drug should be used with considerable caution. In addition, since bacitracin is as effective topically as penicillin, consideration should be given to its use because of its low incidence of sensitization following application.

Absorption and Excretion. When penicillin is injected intravenously, concentration in the blood reaches a "peak" almost immediately. The concentration in the blood decreases rapidly and after a few hours little, if any, can be detected by assay, although it may be detected in the urine for a longer period of time. The peak concentration of penicillin following intravenous injection as well as the duration of blood concentrations depends on the quantity of the drug administered. Since the first clinical trials were made in this country, it has been customary to consider that a maintained plasma concentration of 0.03 unit per ml. or better is satisfactory for most penicillin-susceptible infections other than the most severe. This is an arbitrary figure and it was chosen primarily because of limitations of the bio-assay method utilized for determining blood concentrations of penicillin in man. Obviously, some bacterial strains are inhibited by lower concentrations of the drug while others require considerably higher ones to obtain the desired therapeutic result. Practically all of the clinical evidence available on penicillin is based on a maintained plasma penicillin concentration and for general use this appears to be the most satisfactory procedure. There is, however, both laboratory and clinical evidence that a continuous plasma concentration is not essential for good therapeutic results.

After intramuscular injection, the highest penicillin concentration in the blood is reached usually within one-half hour with a slower fall than seen following intravenous administration. The peak concentration of penicillin in the blood is not as high as would occur with the same dose

given intravenously. For intramuscular injection, the intermittent method is most generally used although under certain exceptional circumstances the drug is given by continuous intramuscular infusion. Subcutaneous injection is apt to be painful, and absorption is slow and uncertain. It has been shown that radio-active penicillin, injected intramuscularly, is not stored in the body but is quickly excreted as penicillin or penicillin-decomposition products.

Early clinical trials with oral therapy were for the most part disappointing, and it was felt that this was due to inactivation of the drug by gastric secretions. During recent years, several investigators have shown that the oral route is effective for the treatment of many infections, provided sufficiently large doses are administered at proper mid-meal periods. It is generally accepted that approximately five times as much penicillin is required orally as intramuscularly to obtain comparable plasma concentrations of the drug. These large doses are necessary because of the inactivating effect of gastric secretions and food, incomplete absorption, and inactivation by penicillinase-producing bacteria in the intestinal tract.

Penicillin may be administered by inhalation as an aerosol of finely micronized dust and by instillation in solution into the pleural, pericardial, peritoneal, or synovial spaces. By these methods of administration, a local or topical effect of the drug is obtained and although absorption may occur, it is erratic and unreliable where systemic therapy is needed. Soluble tablets of penicillin (containing nothing but the drug) are available for sublingual use. Satisfactory absorption by this route has been reported and this mode of administration may have a place in the treatment of patients who have difficulty in swallowing the larger penicillin tablets.

Diffusion of penicillin from the circulation into the tissues depends on the plasma concentrations, and the diffusion into the tissues will proceed as long as the concentration of diffusible penicillin in the plasma exceeds that in the tissues. The drug diffuses readily from maternal to fetal blood and into the abdominal cavity. Some diffusion also occurs into the pleural, pericardial, and synovial fluids, and bile. Very little of the drug penetrates into abscess cavities, brain and nerve tissues, bone, chambers of the eye, nor does it appear in any appreciable concentration in sweat, saliva, tears, or milk. Unless large doses are administered, very little diffusion into the cerebrospinal fluid will occur except where the meninges are inflamed.

As indicated above, after penicillin is absorbed most of it is rather

rapidly excreted in the urine. The renal clearance of crystalline penicillin G approximates the total renal plasma flow. It is about four or five times greater than the renal clearance of inulin or sodium thiosulfate. As a matter of fact, the normal renal clearance of penicillin so closely approximates the renal plasma flow that it has been suggested as a possible substitute for para-aminohippuric acid or diodrast as a test for renal plasma flow and renal function. The soluble salts of penicillin are excreted in the greatest amounts during the first hour but diminishing quantities may be found for several hours thereafter.

Because penicillin is absorbed and excreted rapidly, numerous attempts were made to develop pharmaceutical forms of the so-called "repository types" which would allow a slow and more or less constant release of penicillin into the general circulation from a tissue depot. The first of these was a suspension of penicillin in peanut oil and wax. With the development of procaine penicillin, penicillin in oil and wax became less and less popular until at the present time practically none is available commercially. Procaine penicillin either in oil or in aqueous suspension is slowly absorbed from a tissue depot because of its insolubility. This drug has a solubility of only 0.67 per cent. Further prolongation of blood concentrations of procaine penicillin in oil is obtained by the addition of 2 per cent aluminum monostearate. The latter drug has been used in tremendous amounts during the past few years. Obviously the addition of peanut oil, sesame oil, beeswax, and other substances to penicillin increases the possibilities of sensitization. The occasional side reactions obtained with the so-called repository forms of penicillin, however, have not mitigated against their use.

In attempts to prolong the activity of penicillin in the body, agents which compete with penicillin for the same renal excretory mechanism have been proposed. These agents, para-aminohippuric acid, diodrast, and carinamide, act by retarding the excretion of penicillin, thus prolonging its presence in the blood. Recently a new, relatively nontoxic compound, p-(di-N-propylsulfamyl)-benzoic acid[1] has been shown to retard the excretion of penicillin with a daily dose of 2.0 grams (0.5 gram every 6 hours) to the same extent as a daily dose of 24 grams of carinamide (3 grams every 3 hours). This drug also delays the excretion of para-aminosalicylic acid, and because of this is under study in combination with para-aminosalicylic acid in the treatment of tuberculosis.

[1] "Benemid"—trademark.

STREPTOMYCIN AND DIHYDROSTREPTOMYCIN

The speed with which streptomycin was demonstrated to be an effective weapon in the fight against infectious diseases was in part due to the great need for an agent active against the gram-negative organisms and the mycobacteria. Within a year of the announcement of the discovery of this drug, its *in vivo* activity was established and in spite of extremely short supplies, it had been shown clinically to be a safe and effective chemotherapeutic agent. It is the first and only drug of proven value that phthisiologists have had in the treatment of tuberculosis. Unfortunately, it is not the answer to definitive treatment of this disease although there is no question but that it is a most valuable adjunct to other forms of therapy.

From the standpoint of the amounts injected, particularly in the treatment of gram-positive and gram-negative infections, streptomycin may be considered to be a drug of low toxicity. In the process of the development of streptomycin, the organism *Streptomyces griseus* produces substances in addition to the antibiotic. Among these substances are histamine and histamine-like substances which in the early impure lots were carried over into the finished drug. These histamine-like substances were responsible for the reports of nausea, vomiting, headache, and flushing of the face following parenteral use of the drug. *Streptomyces griseus* not only produces unrelated chemical compounds which cause untoward reactions on injection, but produces also vitamin B_{12} and several other growth-promoting substances during the fermentation process.

Pyrogenic substances are produced in every batch of streptomycin, or for that matter in every batch of any antibiotic produced by the fermentation process. These pyrogens result from breakdown of the cell of the micro-organism, thereby releasing material which on injection raises the body temperature. The elimination of pyrogens from antibiotics has been a problem since their first use in man. It will be remembered that one of the first side reactions of penicillin was a pyrogenic response in man. Present-day penicillin rarely, if ever, contains pyrogenic substances since they are eliminated in the process of manufacture. Similarly, these substances are rarely found in present-day commercial lots of streptomycin. The presence of histamine-like substances and pyrogens in dihydrostreptomycin has been rarely noted also since in the case of this drug its clinical use came about only after relatively pure streptomycin was available. Some evidences of renal irritation occasionally accompanied by impairment of renal function have been reported, particularly with early lots of strep-

tomycin. Similarly, intramuscular administration of partially purified streptomycin gave rise to some degree of soreness and induration at the site of injection. As the purity of the drug increased, however, reports of renal irritation and impairment of renal function decreased in number. It should be emphasized in any case that impairment of renal function occurred in most instances during therapy in the presence of a probable renal disease which had existed before streptomycin treatment was instituted. There appears to be no significant evidence that dihydrostreptomycin produces renal impairment with the ordinary dosage regimens.

There is some evidence that streptomycin hydrochloride is more irritating than streptomycin sulfate on intramuscular administration, and this irritation seems to be related to the hydrochloride portion of the molecule. The apparent greater toxicity of the hydrochloride salt of streptomycin has been sufficient to result in the elimination of this salt of streptomycin from the market, so that at the present time streptomycin sulfate and streptomycin calcium chloride trihydrochloride are the only salts available for clinical use. The only dihydrostreptomycin salt available is the sulfate salt.

In a general way, the toxicity of dihydrostreptomycin is similar to that of streptomycin but this toxicity should be compared with relatively pure streptomycin only since dihydrostreptomycin is produced from relatively pure lots of streptomycin. The neurotoxicity of dihydrostreptomycin when tested in animals is approximately one half that of streptomycin. The lower neurotoxicity of dihydrostreptomycin has been shown clinically in man by several investigators. A delayed neurotoxic effect of dihydrostreptomycin on the auditory system of patients evidenced by delayed deafness several weeks after treatment had been discontinued has also been reported. There is some question as to whether this "delayed deafness" is in reality delayed or whether it is actually a slow progressive increase in deafness. Studies are now in progress in an attempt to determine the facts in this unexpected toxic reaction in a relatively large group of patients. In any case, careful investigation has failed to demonstrate a greater neurotoxicity of dihydrostreptomycin over streptomycin on the auditory system in cats. Indeed, in these animals dihydrostreptomycin appears to affect the auditory and vestibular systems to a lesser degree than does streptomycin.

As with the other antibiotic drugs, streptomycin causes skin eruptions during the course of treatment. These are relatively common but usually are transient and not considered to be an indication for the discontinuance of therapy. Although the impurities present in the early crude preparations of streptomycin may have been responsible for some of the skin reactions

observed, the drug is capable inherently of producing sensitivity. Skin reactions occur in about 4 per cent of the cases treated. Occasionally severe urticaria complicating streptomycin therapy occurs, and two deaths have been reported from dermatitis and stomatitis during streptomycin therapy. Contact dermatitis is caused by streptomycin more often than by penicillin. Not infrequently, individuals in industrial plants handling the drug become sensitive to it. In addition, nurses having intimate contact with the drug often show epidermal sensitization to streptomycin.

Absorption and Excretion. Following injection, streptomycin is readily absorbed and excreted. The usual method of administration is by the intramuscular route, and although intravenous administration has been used, it is no longer recommended, not only because of inconvenience to the patient but also because constant high blood concentrations are unnecessary. This is particularly true in the treatment of tuberculosis where prolonged high concentrations of the drug are not essential and in addition increase the possibility of neurotoxic reactions. The rapid disappearance of streptomycin from the blood can be accounted for by its early appearance in the urine. Following oral administration of the drug in animals relatively small amounts are found in the blood. This lack of absorption of streptomycin from the gastro-intestinal tract is responsible for the low blood concentrations and is consistent with the large amount of drug recovered in the feces. In general, these results are similar to those found following oral use of streptomycin in man. The drug may be concentrated and excreted in the bile. It passes the placental barrier and is readily available to the fetal circulation.

Streptomycin cannot be demonstrated in the blood serum of patients following the administration of 0.5 gram per day by nebulization or by mouth and, furthermore, urinary excretion of streptomycin under these conditions is negligible. Injections of from 50,000 to 100,000 μg. per day intrathecally for meningitis show from 6 to 50 μg. per ml. in the spinal fluid 12 hours later. No significant amounts of streptomycin appear in the spinal fluid following intramuscular or subcutaneous injections of from 50,000 to 200,000 μg. In patients with tuberculous meningitis receiving streptomycin by continuous subcutaneous infusion for over 48 hours, streptomycin may be demonstrated in the spinal fluid in amounts varying from 4 to 18.5 μg. per ml. It would appear that the presence of an inflammatory reaction in the meninges increases the possibility of diffusion of streptomycin into the spinal fluid. In man, the serum concentration obtained within one hour after intramuscular injection of streptomycin approximates that

obtained by intravenous injection of the same dose indicating a rapid absorption from the muscle. Streptomycin is absorbed more slowly following intramuscular or subcutaneous injection than is penicillin and similarly is excreted at a slower rate. Blood concentrations may be maintained for approximately 12 hours following the injection of 0.5 gram of streptomycin as the sulfate.

Most of the streptomycin injected parenterally is excreted through the kidneys and although rather broad variations in per cent recovery of streptomycin in the urine of man have been reported, it appears that where normal kidney function is involved, between 50 and 60 per cent of the streptomycin administered parenterally is excreted in the urine within a 24-hour period. Excretion of the drug is most rapid in the first four hours while the greater part of the antibiotic is excreted in the urine within the first 12 hours. Obviously in the presence of renal damage, markedly different results may be obtained. The renal clearance in man is approximately 70 per cent of the simultaneously determined glomerular filtration rate. In the dog practically all the streptomycin administered can be accounted for by excretion in the urine. In man, however, a considerable amount of the drug is frequently not found in the urine.

Several daily injections of streptomycin parenterally usually produce an equilibrium between the quantities administered and the amounts excreted. In the individual with normal renal clearance, a balance is reached rather rapidly and, as long as it remains normal, may be maintained for considerable periods of time. In the presence of renal damage, however, the blood concentrations may rise very rapidly. In any case, it is advisable and in some instances essential that the concentration of streptomycin in the serum be followed by laboratory assay. As has been indicated previously, the development of neurotoxic symptoms is related to the height of the blood concentration and duration of treatment. High concentrations of streptomycin in the serum for prolonged periods of time may result in evidences of neurotoxicity.

Bacitracin

Although the bacitracin originally produced was made by the surface culture method, the present material is produced by deep-tank fermentation. Production of this drug in the United States is largely by one manufacturer but approximately five have produced small quantities during the past two years. There appear to be no differences in the antibacterial activity of that produced by surface culture and that produced by deep tank.

There is little doubt concerning the toxicity of the bacitracin now commercially available. It is produced fairly uniformly at a potency of 40 units per mg. and this is approximately 60 per cent pure. Further purification beyond 45 units per mg. results in some instability which becomes pronounced at potencies much in excess of 45 units per mg. Animal tests invariably show the drug at these potencies to be nephrotoxic, producing damage to the renal tubules of mice and rats. Kidney damage is observed in dogs given 5,000 units per kg. twice daily for three days.

In man, bacitracin has been shown to produce local pain and injury on intramuscular injection. At the site of injection, petechiae of the skin and macular rashes have been observed. Some nausea and vomiting have also been noted, and occasionally tinnitus occurs. Of more importance, however, is the appearance of albuminuria and an increase in blood urea nitrogen in some patients. Most patients treated with bacitracin show some nephrotoxic phenomena. These vary in degree with different lots of bacitracin. The nephrotoxicity is reflected in the blood NPN, in the urinary albumin, and in low phenolsulfonphthalein excretion. Abnormal urinary changes are observed after the eighth or tenth injection with daily doses of 40,000 to 80,000 units and after the second injection of a single daily dose of 100,000 units for two days. Evidences of liver damage have not been reported and no significant effects have been noted on erythrocytes or white blood cells.

Absorption and Excretion. Bacitracin is absorbed readily following intramuscular injection but disappears from the blood less rapidly than does penicillin. Intramuscular injection of bacitracin in man produces higher and more prolonged blood concentrations than those observed after penicillin administration. The differences in the blood concentrations of penicillin and bacitracin have been shown to be due to their different rates of urinary excretion. In contrast to the mechanism of excretion for penicillin, bacitracin is cleared by the kidneys at a rate approximating that of glomerular filtration.

Following parenteral injection of bacitracin in animals, the drug is found in the urine, kidney, blood, bile, lung, bone marrow, skin, heart, muscle, skeletal muscle, liver, spleen, cerebrospinal fluid, and brain. In man, bacitracin readily diffuses into the pleural and peritoneal cavities. In general, following large intramuscular injections of this drug in man in the absence of inflamed meninges, bacitracin is not usually recovered in the cerebrospinal fluid.

When 50,000 units or about one-half of the maximum daily dose is

administered intramuscularly, the peak concentration of the drug in the blood develops by the fourth hour, after which the concentration gradually declines. Individual variation in blood concentrations may be quite marked following intramuscular use. Blood concentrations following injection of 50,000 units may vary from 0.03 unit per ml. to 0.8 unit per ml.

The oral administration of bacitracin does not ordinarily result in measurable blood concentrations, the greater portion of the total dose being recoverable in the feces. Following unusually large oral doses or prolonged oral administration, however, a trace of activity may be observed in the blood and measurable amounts found in the urine, indicating that some absorption has occurred.

Bacitracin, after parenteral administration, is excreted mainly by way of the urine. Wide discrepancies exist concerning its excretion. In one experiment 10 to 30 per cent of the injected dose was excreted in the urine within twenty-four hours and this suggested that the greater portion was either retained in the body or inactivated. Others have found that practically 100 per cent of the dose administered could be accounted for by urinary excretion within eight hours. The fact that in one case much smaller doses were used may account for this discrepancy. Under experimental conditions, increasing the dose from 10 to 100 units per kg. results in a slightly decreased renal clearance. This agrees with the observation that after relatively large doses in animals, damage to the tubules of the kidneys occurs rapidly and this damage is reflected in the later excretion rate.

Aureomycin

In aureomycin we have a chemotherapeutic agent that can be used without fear of serious toxic effects. Untoward side effects consist of anorexia, nausea, vomiting, epigastric distress, heartburn, and diarrhea, which occur more often in females than in males. The diarrhea often persists for several weeks but may be helped by the administration of kaolin, pectin, or bismuth. Sometimes patients complain of a bizarre desire for certain types of food and occasionally there is a compelling and voracious appetite for any kind of food. Other side effects that have been reported following aureomycin are stomatitis, cheilosis, skin and mucous membrane eruptions, and vaginitis. In addition, a Herxheimer type of reaction has been observed in cases of brucellosis treated with aureomycin. These conditions usually do not interfere with treatment. Early commercial lots of aureomycin caused more nausea, vomiting, and diarrhea than those now

available. It is certain that, although the drug itself may cause these symptoms in patients, a relatively large number of the early reports of toxic manifestations were due to the presence of impurities. Present-day aureomycin is recrystallized three times and, as a result, a product of extremely high purity is available for clinical use.

The diarrhea observed in patients treated with aureomycin for a period of three or more days may not be a toxic manifestation of the drug, but rather an expression of the profound effect this drug has on the intestinal flora. In this respect it is similar to terramycin or, for that matter, any combination of chemotherapeutic agents that are markedly active against both gram-positive and gram-negative aerobic and anaerobic bacteria of which the intestinal flora are comprised. Oftentimes, patients treated with these broad spectrum antibiotics for a period of three days or more show nothing but pure cultures of yeast in their stools. Not infrequently the stools become loose, lose their normal odor, and the patient complains of pruritus ani. The latter is probably due to the abnormal shift of the bacterial flora to the acid-producing yeasts.

There is a relatively low incidence of vomiting in patients treated with aureomycin and this may be controlled quite readily with alkalies and, in some instances, milk. The use of aluminum hydroxide is not recommended since it interferes with proper absorption.

Although aureomycin is used in large part orally, the intramuscular and intravenous routes of administration have been explored. Intramuscularly, aureomycin causes considerable irritation and is extremely painful. There is no preparation commercially available at this time for intramuscular use. There is, however, an intravenous dosage form. This method of parenteral administration is very valuable, perhaps essential, in certain types of cases. In comatose patients and those unable to swallow, intravenous aureomycin has been used with considerable success. Unfortunately, even when used with the special diluents, glycine and leucine, phlebitis occurs in approximately 10 per cent of patients so treated. This reaction subsides promptly without serious residual effect as soon as this form of medication is stopped. Both nausea and vomiting have been reported following the intravenous use of aureomycin.

Aureomycin retards proliferation of fibroblasts, epithelial cells, and cells from explants of embryonal brain tissue at levels approaching those obtained in patients under treatment with this drug. At concentrations of 1,000 μg. per ml., inhibition of growth of these cells has been obtained.

These toxic manifestations of aureomycin appear to be of little clinical significance.

Aureomycin, like all drugs, will sensitize some individuals. The evidence so far indicates that the incidence of patients who become sensitive to the drug is extremely low. Furthermore, it is rarely necessary to withdraw the drug from those patients who develop reactions to it.

In considering the toxicity of aureomycin, one should not forget its relative toxicity as compared to that of penicillin which for all practical purposes is considered atoxic. The average oral dose of crystalline penicillin G is 100,000 units (0.06 gram) every three hours. A million units of penicillin is 0.6 gram, yet doses of aureomycin as high as 30 grams orally have been used without serious toxic reactions. One must conclude that we have in aureomycin a drug practically free from toxicity.

Absorption and Excretion. Aureomycin is readily absorbed from the intestinal tract when administered orally in capsule form. Following absorption it diffuses into all body fluids and tissues. In single doses of from 0.75 to 1.0 gram, therapeutic serum concentrations are obtained quite rapidly and these are maintained for relatively long periods of time. These serum concentrations are maintained at therapeutic levels for 6 to 8 hours and complete disappearance from the circulation is not observed for from 24 to 30 hours. When doses of 0.5 gram to 1.0 gram are administered to patients every 6 to 8 hours, the serum concentration of the drug is maintained at from 2 to 4 μg. per ml., although the concentrations in some patients may exceed 6 or even 8 μg. per ml. From the circulation, aureomycin readily passes into the peritoneal fluid, spinal fluid, bile, urine, and milk. It passes into the fetal circulation through the placenta and has been found in the liver, kidney, lung, and spleen. Aureomycin probably passes the blood-brain barrier in therapeutic amounts and the presence or absence of inflammation is not a controlling factor as it is in the case of penicillin and streptomycin.

The concentration of aureomycin found in the urine following oral administration of this drug in either single or multiple doses is relatively high. Peak concentrations of from 50 to 250 μg. per ml. of urine are obtained following single doses of from 0.5 to 2.0 grams. For the most part these peak concentrations may be maintained by similar doses given every six hours. The successful use of this drug in certain types of urinary tract infections, particularly those of the bladder, is in large part due to the relatively high excretion rate of this drug.

CHLORAMPHENICOL

Chloramphenicol was the first of the broad spectrum antibiotics to be reported in the medical literature. The generic term "chloramphenicol" was derived from the now known structure of this drug which was first called "Chloromycetin." The latter name, which incidentally is also descriptive of the drug, is the trademark name. Chloramphenicol is unique among the antibiotics. It is the first and only antibiotic ever synthesized on a commercial basis. The production of this drug, which today is measured in tons and produced largely by synthesis, was an extraordinary accomplishment.

Chloramphenicol is a drug of remarkably low toxicity. A review of its clinical history indicates that the great majority of investigators who have used this drug report "no untoward reactions." The nitrobenzene nucleus might suggest toxicity for man, yet in a relatively large number of patients on high dosages, no evidence of intolerance or toxicity has been observed.

Although side reactions such as nausea, headache, skin eruptions, and enteritic symptoms are rare following the use of therapeutic doses of chloramphenicol, occasional untoward reactions, minor in character, have been recorded. In one case there was an altered sense of taste; food lost its savor and smoking was not pleasant. A second side effect was the appearance of a sensitivity response which occurred on the sixth day of treatment. At this time the patient felt somewhat dizzy, his face became flushed, and numerous red macular lesions appeared over his face. The pulse and respiratory rates were accelerated. There was no associated pruritis. These symptoms persisted for about 30 minutes and then subsided spontaneously. The same syndrome occurred after the last dose of drug was given. Stomatitis may be a possible side reaction to chloramphenicol and some looseness of stools or diarrhea may occur. In the treatment of syphilis a mild Jarisch-Herxheimer reaction has been noted. This reaction is perhaps less frequent in patients treated with chloramphenicol than those treated with penicillin. In general, fewer cases of loose stools and diarrhea are reported with chloramphenicol than with either aureomycin or terramycin. An explanation of this, in part at least, may be the rapid absorption of the chloramphenicol from the gut and the rapid inactivation of that remaining in the intestine. In addition, little antibacterial effect is obtained on the intestinal flora in contrast to the rather profound antimicrobic effect of terramycin and aureomycin on these organisms. Yeasts are frequently found to make up a great proportion of the intestinal flora following extended treatment with either terramycin or aureomycin, while chloramphenicol has little or no such effect.

Proliferating epithelial cells and fibroblasts and cells from explants of embryonal brain tissue are retarded in their growth *in vitro* by concentrations of chloramphenicol of 10 μg. per ml. This, of course, is in the range obtained with certain dosage regimens of this drug. The cells are completely inhibited in their growth by concentrations of 1,000 μg. per ml. The latter amount is well beyond the blood concentration of the drug obtained in clinical use. In view of the large amounts of chloramphenicol that have been used clinically in man, it does not appear that its effect on the growth of proliferating cells is of clinical significance.

Absorption and Excretion. Chloramphenicol is very rapidly absorbed from the intestinal tract in man. It is metabolized readily and excreted by way of the kidneys. Present evidence indicates that approximately 90 per cent of the administered dose may be accounted for in the urine in a period of 24 hours, although relatively low concentrations of active chloramphenicol are found. Probably less than 10 per cent of the administered dose is excreted unchanged, while a great bulk of the drug is excreted in the form of an inactive nitro compound.

Although lower concentrations of active chloramphenicol are demonstrated in the urine following the oral use of this drug, the blood concentrations obtained with chloramphenicol are somewhat higher than those obtained following comparable oral doses of terramycin and aureomycin. The concentration of chloramphenicol in the blood drops off rapidly so that by the sixth hour its concentration is somewhat lower than that obtained with similar doses of either terramycin or aureomycin. The more rapid disappearance of chloramphenicol from the blood may be explained by the fact that it is more rapidly metabolized into the inactive nitro compound. In spite of this, however, in some subjects chloramphenicol can be demonstrated in low concentrations at the twenty-fourth hour. In the therapeutic use of the drug, proper dosages at intervals of six to eight hours have been shown to be clinically effective. As with single doses, the blood concentrations obtained are much higher than those obtained with comparable oral doses of aureomycin and terramycin.

Terramycin

The first report on terramycin, the third of the broad spectrum antibiotics to be isolated, gave an indication of its wide clinical possibilities. This drug has an acute and chronic toxicity comparable to the other broad spectrum antibiotics. Although daily intramuscular injections of the drug were well tolerated by dogs for a period of two months or more, it was indicated

that intramuscular use of the drug would probably cause local irritation. In high doses (75 to 225 mg. per kg.) sodium terramycin in dogs resulted in impaired renal and liver functions. Dogs dying from these large intramuscular doses of sodium terramycin showed cloudy swelling of the liver and fatty metamorphosis of the kidneys.

Terramycin, as the hydrochloride, is administered largely in capsule form by the oral route. Patients treated by this mode of administration rarely show untoward reactions although there is evidence that terramycin causes gastro-intestinal irritation in some patients. This is manifested by nausea, vomiting, and diarrhea. This is usually minor in character. The nausea and vomiting can be controlled by giving the capsules with milk. The use of milk does not interfere with the absorption of terramycin; however, the use of aluminum hydroxide gel is contraindicated as it is with aureomycin. Other reactions reported include proctitis, vaginitis, and pruritis, usually of a mild degree.

Terramycin is now available for intravenous administration. This preparation, however, should not be used except in those patients who, for one reason or another, are unable to take the drug by mouth. The relatively high alkalinity of the preparation may cause phlebitis on intravenous administration and patients receiving this type of medication should be given the drug by the oral route as promptly as possible. The low toxicity of terramycin by mouth is evident from the fact that doses of at least 15 grams per day over a period of 10 to 15 days have been given without evidences of serious toxic effects.

Absorption and Excretion. The close relationship between terramycin and aureomycin is readily demonstrable in the absorption and excretion of these drugs following oral administration. Both drugs are readily absorbed from the gastro-intestinal tract and are readily demonstrable in the blood and urine. In addition, appreciable amounts are excreted in the feces and thereby alter the intestinal flora of the patient. As with aureomycin, complete disappearance of terramycin from the blood may occur as late as the twenty-fourth to thirtieth hour after single doses. There is, however, a definite diminution in the amount of terramycin in the blood six to eight hours after single doses of 0.5 to 2.0 grams. In contrast to aureomycin, which is not very stable in solution, terramycin is quite stable in the presence of serum, urine, and other body fluids. Terramycin does not readily traverse the blood brain barrier as aureomycin does although in cases where the meninges are inflamed, terramycin has been demonstrated in the cerebrospinal fluid. Terramycin diffuses readily into the pleural fluid and passes

through the placenta and is present in the fetal circulation. The drug is also excreted readily in the bile. The serum concentrations of terramycin after oral administration are relatively high and prolonged. As the dose is increased from 0.5 to 2.0 grams there is a tendency for the serum concentration to increase correspondingly at any given time. However, the increase in serum concentrations of this drug following single oral doses of from 1 to 2 grams is not quite as great as one would expect, and furthermore the serum content of terramycin is not significantly increased by increasing the size of a single dose from 1 to 3 grams. It appears that only a certain amount of terramycin is absorbed from the circulation regardless of the size of the oral dose. Blood concentrations of terramycin can be maintained at a relatively high level by oral administration every six hours. Following oral administration of 0.25 gram, 0.5 gram, and 1.0 gram given at six-hour intervals for a period of 24 hours, serum concentrations rise sharply with increased dosage.

Urinary excretion of terramycin is relatively high following both single and multiple doses. Indeed, considerably more terramycin is found in the urine than aureomycin after similar doses. The differences in recovery of terramycin and aureomycin in the urine following the same dose do not appear to be fully explained on the basis of the instability of aureomycin in urine.

Summary

Pharmacologically, the clinically useful antibiotics are a unique group of drugs. Penicillin is nearly non-toxic but may cause annoying allergic manifestations especially when given topically. Both streptomycin and dihydrostreptomycin are essentially non-toxic when given over short periods of time, although repeated dermal exposure to either drug may give rise to allergic phenomena, and both drugs may cause vestibular and auditory pathology when given over long periods. The polypeptide antibiotics possess somewhat more toxicity. Tyrothricin and its constituents gramicidin and tyrocidine have a systemic toxicity which precludes their parenteral use or makes their application into body cavities and other sites where absorption is likely undesirable. Bacitracin is nephrotoxic and its parenteral use is justified only under exceptional circumstances. The broad spectrum antibiotics, aureomycin, chloramphenicol and terramycin are essentially non-toxic but they may give rise to sensitization reactions and are apt to induce minor gastro-intestinal disturbances.

As one would expect, the presently employed antibiotics are readily

absorbed following one or more standard routes of administration, are able to diffuse into the various tissues, are considerably more active against the parasitic cells than against the host cells and, once their work has been completed, they are destroyed by normal metabolic processes or are excreted from the body by natural routes.

Many of the other antibiotics are of no clinical value because of their adverse pharmacological activity. These substances, although highly bacteriostatic, are either too toxic for use in man or are slowly absorbed, poorly diffusible or inactive in the body. These properties can only be determined after thorough pharmacological study and serve to emphasize the importance of pharmacological techniques in the final evaluation of any new antibiotic.

Microbiology in Relation to Antibiotics

GLADYS L. HOBBY*

"The component members of any society, whether it be an aggregation of human beings or of other living organisms, are continuously influenced by their environment. This is equally true of the cells comprising a single organism. Furthermore, the neighboring beings or organisms or cells constitute an important part of that environment. Several organisms or cells whether of the same or different kinds cannot long exist in a limited space without affecting and/or being affected by their neighbors. This fact is fundamental in all studies of growth and development whether one is concerned with the morphogenesis and ontogeny of an individual organism or with the growth, development, and decline of mixed populations of plants or of animals. The same principle applies even in the waxing and waning of human civilization."

R. Pratt, Armageddon of the Microbes: A Consideration of Certain Relationships in a Microbiological Society.[1]

IN this year, commemorating the completion of ten years of antibiotic therapy, there is no need to call attention to the important rôle played by antibiotics today in the control of infectious processes. The remarkable antimicrobial activity of these agents, both *in vitro* and *in vivo,* has been well established during this decade. The word "antibiotic," to designate specifically that group of antimicrobial agents produced by micro-organisms and capable of inhibiting the growth of or destroying other micro-organisms, first came into use just ten years ago, and some have believed that the existence of these substances in nature was not recognized prior to that time. One might say more correctly, however, that not until then was their full import recognized.

It has been stated recently that "the art of chemotherapy is as old as civilization; the science of chemotherapy is the child of today."[2] As early as 1850, Parker[3] wrote:

Science differs from art in the same manner that knowledge differs from skill. An artist may enchant us with his skill, although he is ignorant of all scientific principles. A man of science may excite admiration by the extent of his knowledge, though he has not the least skill to perform any operation of art. When we speak of the mechanic arts, we mean the practice of those vocations in which tools, instruments, and machinery are employed. But the science of mechanics explains the principles

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[1] Pratt, R. Armageddon of the microbes: a consideration of certain relationships in a microbiological society. *Texas Rep. Biol. Med.*, 1949, 6, 12–21.

[2] Work, T. S. and Work, E. *The basis of chemotherapy.* New York, 1948, p. 1.

[3] Parker, R. G. *Natural and experimental philosophy.* New York, 1850, p. 6.

on which tools and machines are constructed, and the effects which they produce. Science, therefore, may be defined, a collection and proper arrangement of the general principles or leading truths relating to any subject; and there is this connection between art and science, namely—'A principle in science is a rule in art.'

Herbal remedies, many of which were recorded in the *Book of Herbs* written in 3000 B.C., were in use for many years. In the first century A.D., the Greek author Dioscorides published an herbal which included five hundred medicinal plants. This was accepted as an almost infallible authority throughout the entire Middle Ages. Two English herbals, the *Grete Herbal* printed by Peter Treveris in 1526 and *Banckes Herbal* printed by Richard Banckes in London in 1525, describe "how man may be holpen with green herbes of the garden and weeds of the field as well as by costly receipts of the apothecaries prepared."[4] Certain of these remedies were used for many years before being replaced by more specific therapeutic agents.

The development of bacteriology as a science, the discovery of the etiological agents responsible for many of the infectious diseases, Lister's introduction of aseptic techniques and his revolution of surgical methods, as well as progress in other fields of biology, chemistry, and medicine contributed much to the change in therapeutic methods. As expressed recently by Sir Henry Dale:[5] "The end of the nineteenth century found medicine still a body of largely empirical knowledge, but the richness of the nineteenth century's closing decade in major discoveries, of the kind from which science advanced with a fresh impetus and in new directions, seems to have caused a sudden acceleration of this process to begin about the turn of the century." Slightly more than a decade ago, however, the wisdom of the early physicians and the fact that there might have been a sound basis for their use of herbs and plants in the treatment of disease was little recognized. Recent interest in the natural origin of antimicrobial agents, however, has stimulated re-evaluation of these remedies, and the active components of many of the herbs and plants have been isolated.

The words "antibiosis" and "antibiotic substance" were used originally to designate "antiliving processes" in a broad sense.[6] Santonin, an anthelminthic derived from the plant *Artemisia maritima* and known to Greek, Roman, and Arab medicine, might well be considered among those capable of exerting such an effect, and probably was one of the first "antibiotic substances" manufactured on a commercial scale in this country. Santonin was used widely until the early part of the twentieth century and appeared in

[4] Freeman, M. B. *Herbs for the mediaeval household.* 2nd ed. New York, 1948, p. 10.

[5] Dale, Sir Henry. Advances in medicinal therapeutics. *Brit. med. J.*, 1950, *I*, 1–7.

[6] Waksman, S. A. What is an antibiotic or an antibiotic substance? *Mycologia*, 1947, *39*, 565–569.

materia medica as late as 1919.[7] The bactericidal agent present in cabbage tissues probably complies more fully with our present concept of an "antibiotic substance." Although its antimicrobial activity was described[8,9] only recently, its healing properties have been recognized for many years, as indicated by Cato (234–149 B.C.): "And if there are any bruises it will break it up and heal it if you apply this mashed cabbage."

During the latter part of the nineteenth century, it was observed repeatedly that, when several bacterial species were allowed to grow together on a nutrient agar plate, a clear, sterile zone sometimes developed around the colonies of certain species. Inhibition of growth of one organism by another thus was observed during the earliest days of the development of the science of bacteriology. Even before methods were established for the isolation and cultivation of organisms in pure culture, this phenomenon was observed in mixed culture. It was Garré[10] who, in 1887, recognized that this might be due to the presence of bactericidal substances secreted by certain types of soil bacteria and capable of destroying other micro-organisms. The scientific use of the word "antibiosis" to describe this phenomenon dates from 1889, when Vuillemin[11] expressed the concept as "one creature destroying the life of another in order to sustain its own—one being in unrestricted opposition to the life of the other," and referred to the active agent as an *"antibiote."*

In 1885, Babes[12] expressed the thought: "If the study of mutual antagonisms of bacteria were sufficiently far advanced, a disease caused by one bacterium probably could be treated by another. A further and wider study of this reciprocal action of bacteria may lead to new ideas in therapeutics." Fifty years elapsed before the development of penicillin for the first time offered support to this concept. During this period, innumerable substances derived from one organism and antagonistic to another were described. These were spoken of as lysins, bacteriolysins, bacteriotoxins, bactericidal substances, bacteriolytic agents, antagonistic substances, etc. Because of their selective action on different bacteria, certain of them were utilized for separating one bacterial culture from another, while others, because of their ability to bring about lysis of still other micro-organisms, at times were uti-

[7] *Squibb's materia medica for the physician and the surgeon.* New York, 1919, p. 220.

[8] Sherman, J. M. and Hodge, H. M. The bactericidal properties of certain plant juices. *J. Bact.*, 1936, *31*, 96.

[9] Pederson, C. S. and Fisher, P. The bactericidal action of cabbage and other vegetable juices. *Tech. Bull. N. Y. St. agric. Exp. Sta.*, 1944, No. 273, pp. 1–32.

[10] Garré, C. On antagonists among bacteria. *Z. Bakt.*, 1887, *2*, 312.

[11] Vuillemin, P. Antibiose et symbiose. *Assoc. franç. Avanc. Sci.*, 1889, *2*, 525, 542.

[12] Babes, V. Concurrence vitale des bactéries. Attenuation de leurs propriétés dans des milieux nutritifs modifiés par d'autres bactéries. Tentatives de therapeutique bactériologique. *J. Connaiss. med. Prat. Paris*, 1885, *7*, 321.

lized in the preparation of vaccines. A few were tried clinically, but proved therapeutically inactive.

In 1929, Fleming[13] in a study of staphylococcus variants observed that the colonies of certain staphylococci had become transparent and had undergone partial lysis in the neighborhood of a large colony of a contaminating fungus. Having been interested in the action of lysozyme for some time previously, it is not surprising that Fleming recognized the possible significance of this lytic effect and isolated the fungus, a strain of *Penicillium notatum* capable of producing penicillin. It is of interest that penicillin, the action of which is most strikingly and indeed most readily demonstrated, *in vitro,* by the zones of growth inhibition about antibiotic-impregnated discs placed on nutrient agar inoculated heavily with penicillin-sensitive strains of micro-organisms, should have been recognized first through its lytic action. It was not chance that Fleming recognized the possible significance of his observation; it was, rather, evidence of "Serendipidus" or a "prepared mind."[14] It is doubtful if another investigator, unfamiliar with the action of lytic agents, would have been impressed with the effect produced by this penicillium; it is doubtful, furthermore, if Fleming, had he observed the same phenomenon ten years earlier, would have recognized its potentialities. Even in 1929, however, the time was not ripe for the development of antimicrobial therapy as it is known today. Not until the scope of the sulfonamides had been defined,[15] and the fact that antimicrobial agents of natural origin as well as chemical origin could exert an effect against systemic infections (at least in animals) had been demonstrated by Dubos,[16] and not until the pressure of an oncoming war stimulated research on agents suitable for the control of infectious agents, was the mind prepared for this new approach. Only then did Florey, with the concerted efforts of his associates,[17] succeed in demonstrating the potentialities of penicillin therapy.

The reports by Florey and his co-workers[17,18] on the nature and action of penicillin are classic in that they represent the first instance in which a

[13] Fleming, A. On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. influenzæ. *Brit. J. exp. Path.*, 1929, *10*, 226–236.

[14] Seegal, D. and Seegal, B. C. Chance and the prepared mind. *Diplomate*, 1950, *22*, 125–131.

[15] Domagk, G. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, 1935, *61*, 250.

[16] Dubos, R. J. Studies on a bactericidal agent extracted from a soil bacillus. I. Preparation of the agent. Its activity in vitro. *J. exp. Med.*, 1939, *70*, 1–10. Production of bactericidal substances (tyrocidine and gramicidin) by aerobic sporulating bacilli. *Ibid.*, 1941, *73*, 629–640.

[17] Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J. and Sanders, A. G. Penicillin as a chemotherapeutic agent. *Lancet*, 1940, *2*, 226–228.

[18] Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner A. D., Heatley, N. G. and Jennings, M. A. Further observations on penicillin. *Lancet*, 1941, *2*, 177–180.

complete evaluation of a drug was carried out by a single group of investigators. Attention was called to a fact, now well accepted, that only by a combination of bacteriological, biochemical, and biological methods can a reasonably accurate picture of an antimicrobial or chemotherapeutic agent be obtained. For the first time the potentialities of antimicrobial agents of natural origin in the treatment of systematic infections were established conclusively.

In the ten years just past, penicillin has become an accepted part of modern medicine. Other antibiotics, including streptomycin, bacitracin, polymyxin, neomycin, chloramphenicol, aureomycin, and terramycin, have been discovered and studied extensively. At least five of these have found a place in therapeutic practice. The rapid development of so many antimicrobial substances in so short a period of time no doubt is indicative of the advance in scientific knowledge which has occurred in recent years. It may be considered, furthermore, a true measure of the value of penicillin, for the impetus to develop new antibiotics was derived largely from observation of the results obtained with this agent. It has been stated recently[19] that: "In the light of present day knowledge, it is fortunate indeed that penicillin was the first antibiotic to be discovered by man. Fortunate, first because penicillin possesses a wide spectrum of antibacterial activity against most of the gram-positive and certain of the gram-negative organisms, and second, because of its almost complete absence of toxicity. Had another antibiotic been isolated first—one effective against but a single disease—it is not improbable that the antibiotic concept as it is known today would never have been developed or would have been seriously delayed. The new drug might have been looked upon as merely a specific therapeutic agent for a given disease without any hint whatsoever regarding the potentialities of the antibiotic approach." That other antibiotics were discovered before penicillin must be admitted, but the remarkable activity of penicillin unquestionably served to emphasize the potentialities of antibiotic therapy in general. The announcement of streptomycin within a few years after the introduction of penicillin, furthermore, emphasized the significance of these agents in antimicrobial therapy and stimulated the search for additional ones.

The search for new antimicrobial agents has been carried out diligently and the claim has been made that essentially every known bacterium, actinomycete, and fungus has been examined for evidence of antibiotic formation. Large-scale systematic studies of the organisms residing in soil from every part of the world, in manure piles, and in polluted water have been

[19] Smith, L. W. Antibiotics and their increasing specificity. *C.S.C. Reporter*, 1949, *6*, 1.

in progress for several years to determine their abilities to produce antimicrobial substances of potential chemotherapeutic value. Similar studies are in progress to determine the existence of such agents in higher plants. Antimicrobial substances from bacteria and fungi may be evaluated with considerable ease, for large quantities may be produced readily. This is more difficult with the antimicrobial agents from higher plants, yet it is known that these substances do exist throughout the plant kingdom.

The past decade has seen not only the development of these so-called "screening programs," but the development of new concepts with regard to the cultivation of micro-organisms for the production of substances such as the antibiotics. That micro-organisms are capable of producing certain chemicals, as, for example, citric acid and riboflavin, more economically than they can be produced synthetically by man, and that micro-organisms at times are capable of synthesizing compounds which to date have never been made by chemical means, is well recognized. The experience with penicillin and other antibiotics has brought abundant confirmation of these facts.

It is now well known that a single substance may be produced by more than one organism, and that a single micro-organism may produce a given antibiotic in more than one chemical form, or may produce two or more completely unrelated antibiotics, depending upon environmental conditions. Variations in the environmental conditions may alter materially the capacity of a given organism to produce an antibiotic, while certain organisms may produce substances capable of inhibiting the activity of antimicrobial agents produced by other micro-organisms.

Penicillium notatum, Penicillium chrysogenum, and *Aspergillus flavus,* for example, all are capable of producing penicillin. Some strains produce more penicillin when used as quiet surface growers and others when used under submerged and aerated conditions. From the original strain of *Penicillium notatum* received from Fleming in 1941, substrains were isolated which produced somewhat higher quiet surface yields than did the original strain; still higher yields were obtained from a strain of *Penicillium chrysogenum* isolated in 1944, while an X-ray mutant of this strain yielded as much as 500 units per ml. and a subsequently developed ultra violet mutant produced from 800 to 1000 units per ml.[20] Through culture work alone, therefore, the potency of penicillin fermentation liquors was increased a hundred fold. Although other developmental factors also

[20] Backus, M. P., Stauffer, J. F. and Johnson, M. J. Penicillin yields from new mold strains. *J. Amer. chem. Soc.*, 1946, *68*, 152–153.

played a part in raising the over-all production, the results from these cannot be compared with the great strides made through culture mutation and selection.

Of special interest, however, are the studies on the optimum composition of media for the production of penicillin. From corn steep liquor, a substance capable of stimulating the development of penicillin by strains of *Penicillium notatum*, the decarboxylation product of phenylalanine was isolated in 1944.[21] This proved to be Beta-phenylethylamine which, when added to culture media, served to increase markedly the output of penicillin. This led to the use of phenylacetic acid and derivatives, a series of compounds which served a double purpose in that they not only increased the potency of fermentation liquors, but also served as precursors for the formation of penicillin G, the species of penicillin later found to be the most desirable therapeutically. The use of precursors has become an established procedure, and more than two hundred different species of penicillin have been developed. Each species differs from the others quantitatively, but not qualitatively, in biological activity; the most commonly recognized species are penicillins G, X, F, dihydro-F, K, and O. It is not uncommon for other antibiotics also to occur in more than one form, and two or more species of streptomycin, polymyxin, neomycin, or bacitracin may be produced by single strains of micro-organisms. These factors have served to complicate the study of antibiotics in general, and only after complete purification, therefore, has it been possible to determine their true nature and action.

The remarkable activity of penicillin, combined with its low degree of toxicity, permitted its use clinically in an extremely impure form. Indeed, over 95 per cent, by weight, of the early preparations consisted of impurities. Even the preparations of streptomycin in use in 1945 were only 25 to 50 per cent pure. Production of penicillin and streptomycin during the early development of each of these antibiotics was hampered, therefore, by lack of a chemical assay method, and it was necessary to develop biological means of determining the amount of active antibiotic present. Serial dilution assay methods were introduced in 1940 and served a useful purpose. Nonetheless, they were inadequate for quantitative measurement of activity and soon were replaced by the Oxford cup plate method, introduced by Heatley and his associates,[22] which measured the activity of a preparation

[21] Mead, T. H., Stack, M. V. and Stewart, D. D. Isolation of the possible penicillin precursors B-phenylethylamine and B-p-hydroxyphenylthylamine from corn steep liquor. *O.S.R.D. Report No. Pen 138* (Oct. 3, 1944), 1–3.

[22] Garrod, L. P. and Heatley, N. G. Bacteriological methods in connection with penicillin treatment. *Brit. J. Surg.*, 1944, *32*, 117–118.

in terms of the size zones of growth inhibition produced by known quantities of antibiotic, as compared with a standard. The original definition of a unit of penicillin, based on this assay procedure, was "the amount of penicillin which when dissolved in 1 ml. of water gives the same inhibition as (a certain partially purified) standard (solution)." Subsequently the unit was redefined, but the method of assay has remained in use. One unit of penicillin is now accepted[23] as "the penicillin activity contained in 0.6 mcg. of the master standard, a specific preparation of crystalline sodium penicillin G." The Oxford cup plate method of bio-assay is remarkably accurate, the error being not more than 5 per cent if carefully controlled experimental conditions and a sufficient number of replicate samples are used. This technique is today the official test for penicillin, despite the fact that all penicillin is produced as crystalline salts and chemical methods of assay are available. It is widely used also for other antimicrobial agents, although the potencies of some are determined by means of a turbidimetric method[24] based on procedures established for the microbiological assay of vitamins. That the value of any antibiotic resides in its antimicrobial activity has remained fundamental, and a bio-assay which measures the antimicrobial power of an antibiotic is considered essential.

The mechanisms by which antimicrobial agents function and the reasons why certain of them are effective against some micro-organisms, while others are effective only against other micro-organisms, is of great interest to microbiologists. This interest is due partly to a belief that an understanding of the factors responsible for their action may result in development of better chemotherapeutic agents. To date, few studies have been undertaken to determine the mode of action of these agents; these few, however, have concerned themselves primarily with penicillin and streptomycin.

Penicillin is an organic acid which is active in low concentrations against many gram-positive and a few gram-negative micro-organisms. It may exert either a bactericidal or bacteriostatic effect depending upon the experimental conditions. Early studies[25] indicated that, under the influence of high concentrations of penicillin, the number of organisms decreases at a constant rate until about 99 per cent of them have been destroyed. The rate of killing varies with different organisms and with the concentration

[23] Federal Register and the Code of Regulations (U. S. Federal Security Agency, Food and Drug Administration), Title 21, Part I, Section 146.1.

[24] McMahan, J. R. An improved short time turbidimetric assay for penicillin. *J. biol. Chem.*, 1944, *153*, 249–258.

[25] Hobby, G. L., Meyer, K. and Chaffee, E. Observations on the mechanism of action of penicillin. *Proc. Soc. exp. Biol., N. Y.*, 1942, *50*, 281–285.

of penicillin used. Its action is specifically inhibited by penicillinase, an enzyme derived from certain resistant gram-negative species. Penicillin is effective only when active multiplication takes place. In view of the fact that penicillin approaches the perfect chemotherapeutic agent more nearly than any other substance known, these observations have been used as a basis for comparison of other compounds.

It was recognized early in the study of penicillin that not only micro-organisms belonging to different species, but also strains within the same species may differ in their sensitivity to penicillin. Determination of the "sensitivity" of a given organism to a specific antimicrobial agent thus became an accepted technique and the "sensitivity" of an organism to an antibiotic was defined as the minimal amount of antibiotic necessary for inhibition of growth. This may be measured by absence of turbidity in a liquid medium,[26, 27] or by the size of zones of growth inhibition surrounding antibiotic-impregnated paper discs placed on heavily inoculated plates.[28]

Streptomycin, an antibiotic produced by an actinomycete, *Streptomyces griseus,* and first announced by Schatz, Bugie, and Waksman in 1944,[29] was the second antimicrobial agent of biological origin to prove successful as a chemotherapeutic agent in man. Streptomycin, in contrast to penicillin, is an organic base which is effective in low concentrations and is active against many of the gram-negative micro-organisms and the acid-fast bacilli. It is predominantly bacteriostatic in nature, although bactericidal activity may be demonstrated under appropriate conditions. The activity of streptomycin is influenced by many more variables than the activity of penicillin, and it is recognized that, other environmental factors being constant, the activity of streptomycin varies markedly with the concentration of antimicrobial agent used, the size of inoculum, the medium and pH. Streptomycin is most effective against rapidly dividing cells, but is capable also of exerting some effect against organisms in the stationary phase.[30, 31, 32]

[26] Hobby, G. L., Meyer, K. and Chaffee, E. The activity of penicillin in vitro. *Proc. Soc. exp. Biol., N. Y.,* 1942, *50,* 277–280.

[27] Dawson, M. H., Hobby, G. L., and Lipman, M. O. Penicillin sensitivity of strains of non-hemolytic streptococci isolated from cases of subacute bacterial endocarditis. *Proc. Soc. exp. Biol., N. Y.,* 1944, *56,* 101–102.

[28] Bondi, A., Jr., Spaulding, E. H., Smith, D. E. and Dietz, D. C. A routine method for the rapid determination of susceptibility to penicillin and other antibiotics. *Amer. J. med. Sci.,* 1947, *213,* 221–225.

[29] Schatz, A., Bugie, E. and Waksman, S. A. Streptomycin, an antibiotic substance active against both gram-positive and gram-negative bacteria. *Proc. Soc. exp. Biol., N. Y.,* 1944, *55,* 66–69.

[30] Lenert, T. F. and Hobby, G. L. Observations on the action of streptomycin in vitro (I). *Proc. Soc. exp. Biol., N. Y.,* 1947, *65,* 235–242.

[31] Hobby, G. L. and Lenert, T. F. Observations on the action of streptomycin in vitro (II). *Proc. Soc. exp. Biol., N. Y.,* 1947, *65,* 242–249.

[32] Henry, R. J. and Hobby, G. L. The mode of action of streptomycin. In: *Streptomycin.* Ed. by S. A. Waksman. Baltimore, 1949, pp. 197–218.

Chloramphenicol is a neutral compound which was isolated originally from cultures of a new species of streptomyces, now known as *Streptomyces venezuelae,* by a relatively simple extraction procedure.[33] It was crystallized at an early stage in its investigation and subsequently synthesized. Chloramphenicol has the formula C_{11} $H_{12}Cl_2$ NO_5 and is optically active; only the d-threo isomer is biologically active. *In vitro* and *in vivo* studies have indicated identical properties for chloramphenicol produced by fermentation and by biological synthesis. Chloramphenicol is highly active *in vitro* against a wide variety of micro-organisms. Its antirickettsial properties and its action against the Salmonella group of organisms, however, are of greatest importance.

Aureomycin, like chloramphenicol, also is derived from a mold belonging to the actinomycete group; it was discovered by Duggar[34] during a study of the antibiotic substances produced by such micro-organisms. The actual constitution of aureomycin has not been reported to date. It is an amphoteric substance, however, which, like chloramphenicol, contains chlorine. It is active against many strains of rickettsiae and against the gram-positive and gram-negative micro-organisms.

Terramycin, the newest of the broad spectrum antibiotics, likewise is produced by a mold belonging to the group of actinomycetes, namely *Streptomyces rimosus.*[35] Originally isolated from the soil, this organism was so named because of its cracked appearance when grown on the surface of solid media. Terramycin is amphoteric in nature, and crystalline acid and basic salts may be prepared; it has a molecular weight of about 460. Terramycin and its salts are effective against a wide variety of aerobic and anaerobic, gram-positive and gram-negative micro-organisms, the rickettsiae, and certain of the spirochaetes and viruses. It exerts an indirect action, furthermore, against certain of the protozoa.

Little has been published on the mechanism of action of aureomycin and chloramphenicol. Indeed, little is known concerning the mode of action of any of the antimicrobial agents. Terramycin, nonetheless, acts promptly upon sensitive cells and exerts a bactericidal effect when the ratio of the concentration of antibiotic to the initial number of organisms is high. As the ratio diminishes, the bactericidal effect likewise diminishes, and only bacteriostatic action may be demonstrated. Under conditions suitable for

[33] Ehrlich J. Chloromycetin, a new antibiotic from a soil actinomycete. *Science,* 1947, *106,* 417.

[34] Duggar, B. M. Aureomycin, a product of the continuing search for new antibiotics. *Ann. N. Y. Acad. Sci.,* 1948, *51,* 177–181.

[35] Finlay, A. C., Hobby, G. L., P'an, S. Y., Regna, P. P., Routien, J. B., Seeley, D. B., Shull, G. M., Sobin, B. A., Solomons, I. A., Vinson, J. W. and Kane, J. H. Terramycin, a new antibiotic. *Science,* 1950, *111,* 85.

bacteriostasis, there is often a prolonged lag period during which no antimicrobial activity is apparent.[36]

The development of these three agents, chloramphenicol, aureomycin, and terramycin has resulted in a marked change in our thoughts concerning the action of antimicrobial agents of natural origin. Although the effect of streptomycin in the treatment of infections such as tularemia and brucellosis had suggested that antibiotics might exert an effect on predominantly intracellular infections, the studies with the broad-spectrum antibiotics afforded the first real evidence to this effect. They illustrated for the first time the fact that antimicrobial agents may be found which will act on intracellular parasites such as the rickettsiae and the larger viruses. In view of these results, it is not too much to assume that ultimately other antibiotics may be discovered which will act on the smaller viruses.

Bacitracin and polymyxin fall into a somewhat different category. They are antimicrobial agents produced by bacterial species, and, like many other antibiotics produced by this group of organisms, are polypeptides which possess a degree of toxicity that to date has limited their use by the parenteral routes of administration. They are capable, nonetheless, of inhibiting growth of a wide variety of micro-organisms.

One cannot begin to mention all of the other antimicrobial agents which have been studied to date. Suffice it to say that they are numerous. A new antimicrobial agent, however, must be better than those mentioned above, it must offer new advantages if it is to gain in importance as a chemotherapeutic agent. The real problem today is to determine which of the presently available antibiotics is of greatest value for the specific purpose at hand.

Certain of the antibiotics have been handicapped by the fact that micro-organisms initially sensitive to them ultimately may become highly resistant after contact with them. This has been described aptly by Garrod[37] as "a path of escape from the suppressive influence of the drug which has been discovered by some species, often with inconvenient and disastrous results." The development of resistance to "antiliving" agents was first demonstrated, toward the end of the nineteenth century, when it was observed that certain bacteria and protozoa could be trained to grow in concentrations of antiseptics and toxic substances that would be fatal to untrained

[36] Hobby, G. L., Lenert, T. F., Pikula, D., Kiseluk, M. and Hudders, M. E. The antimicrobial action of terramycin. *Ann. N. Y. Acad. Sci.*, 1950, *53*, 266–276.

[37] Garrod, L. P. The relations of bacteria to chemotherapeutic agents. (The Kettle Memorial Lecture, delivered at St. Bartholomew's Hospital Medical College on November 22, 1950.) *Brit. med. J.*, 1951, *1*, 205–210.

cells.[38–40] The biochemical and clinical importance of this phenomenon was not recognized, however, until the development of the sulfonamides, and later, streptomycin. That essentially 93 per cent of individuals receiving streptomycin for periods of four weeks or longer show micro-organisms resistant to streptomycin is well known. This is in marked contrast to what is observed with penicillin, for the incidence of emergence of organisms resistant to penicillin is low, and indeed difficult to demonstrate.

It is difficult to say at this time if the development of resistance is an induced response to an external stimulus, or evidence of selection of a spontaneous variant which outgrows the parent under the changed conditions. Although both mechanisms undoubtedly are involved, the weight of evidence, insofar as antimicrobial agents are concerned, possibly favors the latter mechanism, and there is little doubt that Darwinian principles are invoked in the process.[41] The form of resistance acquired through one or both of these two possible mechanisms must not be confused, however, with that form of resistance which occurs naturally.

One of the most remarkable observations in recent years is that made by Miller and Bohnhoff in 1947.[42] These investigators demonstrated that bacteria may become dependent on streptomycin for growth. From each of eighteen strains of meningococcus they obtained two streptomycin-resistant variants. One appeared in small and equal numbers on all concentrations, grew in large yellowish colonies on streptomycin-free and streptomycin-containing media, and retained the original virulence for mice possessed by its parent strain. The other appeared in greatest numbers on concentrations between 100 and 400 mcg. of streptomycin per ml. Its colonies varied in size and color depending upon the concentration of streptomycin in which they were developed, and the organisms were dependent on streptomycin for multiplication. This dependency was demonstrable not only *in vitro* but also *in vivo,* since the organism exhibited no virulence for mice unless streptomycin was administered to the animals following infection. Subsequently, it was demonstrated by other investigators[43] that these changes are specific, and that an organism actually dependent upon streptomycin is incapable of multiplying *in vitro* or *in vivo*

[38] Kossiakoff, M. G. De la propriété que possèdent les microbes de s'accommoder aux milieux antiseptiques. *Ann. Inst. Pasteur,* 1887, *1,* 465.

[39] Massart, J. Sensibilité et adaptation des organismes à la concentration des solutions salines. *Arch. Biol., Paris,* 1889, *9,* 515.

[40] Abbott, A. C. Corrosive sublimate as a disinfectant against Staphylococcus pyogenes aureus. *Johns Hopk. Hosp. Bull.,* 1891, *2,* 50.

[41] Tillett, W. S. The antibiotic age. *Amer. J. Med.,* 1948, *4,* 159–162.

[42] Miller, C. P. and Bohnhoff, M. Two streptomycin-resistant variants of Meningococcus. *J. Bact.,* 1947, *54,* 467–481.

[43] Rake, G. Streptomycin as an essential nutrilite. *Proc. Soc. exp. Biol., N. Y.,* 1948, *67,* 249–253.

in the absence of N-methyl-L-glucosaminido-streptosido-streptidine or one of its derivatives.

One may demonstrate not only organisms which are dependent upon streptomycin for growth, but also organisms whose growth is enhanced by the presence of streptomycin. Two strains of *M. tuberculosis,* the growth of which was enhanced by but was not dependent upon the presence of streptomycin, were isolated by Spendlove and his associates[44] from human sputa, a fact indicating that these variants can exist *in vivo* as well as *in vitro.* That dependent cells are normally present among otherwise sensitive cells was demonstrated by Iverson and Waksman[45] who, in a study of the distribution of dependent cells of *E. coli* in a broth culture of this organism, observed that one dependent cell is present among each 1.5 billion normal sensitive cells. This is in agreement with the earlier observations of Demerec,[46] Alexander,[47] and others to the effect that penicillin-resistant and streptomycin-resistant cells occur normally in exceedingly small numbers among large populations of sensitive organisms.

The emergence of strains resistant to a given antibiotic, after contact with the same substance, is not characteristic of streptomycin alone, but occurs with varying degrees of frequency and at varying rates with a number of different antimicrobial agents. Whether or not the possible appearance of antibiotic-dependent strains is characteristic of streptomycin alone remains to be determined. Nonetheless, these phenomena arouse interest in mechanisms whereby the emergence and/or growth of resistant or dependent organisms may be suppressed.

Ungar in 1943[48] was the first to report the synergistic action of two antimicrobial agents, namely, sulfapyridine and para-aminobenzoic acid, on penicillin. Similar results with penicillin and other members of the sulfonamide group were reported subsequently by Hobby *et al.,*[49] Bigger,[50] and others. With the discovery of the many new antibiotics, interest in the

[44] Spendlove, G. A., Cummings, M. M., Fackler, W. B., Jr. and Michael, M., Jr. Enhancement of growth of a strain of M. tuberculosis (Var. hominis) by streptomycin. *U. S. Public Health Reports,* 1948, *63,* 1177–1179.

[45] Iverson, W. P. and Waksman, S. A. Use of streptomycin-dependent strains of bacteria for demonstrating the ability of microorganisms to produce streptomycin. *Science,* 1948, *108,* 382–383.

[46] Demerec, M. Production of staphylococcus strains resistant to various concentrations of penicillin. *Proc. nat. Acad. Sci., Wash.,* 1945, *31,* 16–24.

[47] Alexander, H. E. and Leidy, G. Mode of action of streptomycin on type b H. influenzae. I. Origin of resistant organisms. *J. exp. Med.,* 1947, *85,* 329–338.

[48] Ungar, J. Synergistic effect of para-aminobenzoic acid and sulphapyridine on penicillin. *Nature,* 1943, *152,* 245–246.

[49] Hobby, G. L. and Dawson, M. H. The effect of sulfonamides on the action of penicillin. *J. Bact.,* 1946, *51,* 447–455.

[50] Bigger, J. W. Synergic action of penicillin and sulphonamides. *Lancet,* 1944, *2,* 142–143.

combined use of these agents increased, and Romansky *et al.*[51] have demonstrated synergistic effects when streptomycin, penicillin, aureomycin, and chloramphenicol were used in pairs. Spicer[52] has obtained similar results with these antibiotics and with bacitracin, while Welch and his associates[53] have demonstrated a more complete elimination of the bacterial flora of the intestinal tract in man with a streptomycin-bacitracin-polymyxin combination. The lack of truly precise methods for determination of the sensitivity of organisms *in vitro* and for measurement of the minimum effective dose *in vivo* has made the laboratory evaluation of the significance of the combined action of antibiotics difficult. Nonetheless, the use of streptomycin in combination with para-aminosalicylic acid in the treatment of tuberculosis and in combination with penicillin in certain other infections has become accepted clinical practice and there can be little doubt of the value of these combinations.

The recent observations of Jawetz *et al.*[54] in this regard were truly unexpected. In mice infected with *Streptococcus hemolyticus* and treated with penicillin and chloramphenicol, he demonstrated that antagonism of one antibiotic for another may occur *in vivo*. Subsequently, Bigger,[55] who demonstrated synergism with all but one of the fifteen combinations of sulfathiazole, penicillin, streptomycin, chloramphenicol, boric acid, and para aminosalicylic acid, also reported antagonistic action with five of these combinations. More recently, Lenert *et al.*,[56] working with *Mycobacterium tuberculosis*, reported synergism in 93 of 132 combinations, while they noted an antagonistic effect in two instances only. The observations of these investigators confirmed, with this species of micro-organism, those of Jawetz and his associates to the effect that chloramphenicol is capable of exerting an apparent antagonistic effect against certain other antibiotics. Whether or not this represents a true antagonism of the antibiotic, or only a stimulation of growth of the organism by the use of subinhibitory amounts of chloramphenicol, remains to be determined. No data are avail-

[51] Romansky, M. J., Fusillo, M. and Levy, M. The synergistic action of combinations of the antibiotics and their practical application. *Trans. 9th Strep. Conf.*, 1950, 173–174.

[52] Spicer, S. Bacteriological studies of the newer antibiotics: Effect of combined drugs on microorganisms. *J. Lab. clin. Med.*, 1950, *36*, 183–191.

[53] Welch, H., Randall, W. A. and Price, C. W. The effect of streptomycin-bacitracin-polymyxin combination, polymyxin B and streptomycin with glucuronolactone on the intestinal flora of man. *J. Amer. Pharm. Ass., Sci. Ed.*, 1950, *39*, 486–489.

[54] Jawetz, E., Gunnison, J. B. and Coleman, V. R. The combined action of penicillin with streptomycin on chloromycetin on enterococci in vitro. *Science*, 1950, *111*, 254–256.

[55] Bigger, J. W. Synergism and antagonism as displayed by certain antibacterial substances. *Lancet*, 1950, *2*, 46–50.

[56] Lenert, T. F., Dealy, C., Schneider, E. and Hobby, G. L. The combined action of antimicrobial agents against Mycobacterium tuberculosis; presented before the N. Y. Branch of the Soc. of Amer. Bacteriologists, Jan. 4, 1951.

able to date to indicate that subinhibitory amounts of chloramphenicol are capable of acting as a growth stimulant for micro-organisms, although it has been demonstrated recently by Garrod[57] that penicillin is capable of exerting such an effect.

One cannot help but wonder what may be the relationship, if any, between the growth-stimulatory effect of subinhibitory amounts of antibiotics on micro-organisms and on higher animals. McGinnis *et al.* in 1949[57] reported a marked increase in the growth of turkey poults fed a diet supplemented with a whole dried aureomycin fermentation mash. This response was obtained under conditions where vitamin B12 was ineffective in promoting growth. Subsequently, Stokstad *et al.*[58] demonstrated that a similar product gave increased chick growth over that obtained with crystalline vitamin B12, while these investigators and others[59,60] reported similar growth-promoting effects in chicks, turkeys, and pigs with crystalline aureomycin and streptomycin. That penicillin and bacitracin likewise exhibit this effect has been shown by Whitehill and his associates[61] and by Sieburth *et al.*,[62] respectively. Reynolds *et al.*[63] described recently a similar stimulation of growth due to terramycin, and it is apparent that many, if not all, antibiotics may possess this unusual property. Although crystalline antibiotics, free of vitamin B12, are capable of producing stimulation of growth, nonetheless, optimum responses are obtained only in the presence of this vitamin.[63] Experiments conducted by Whitehill *et al.*,[61] in which no response was apparent when penicillin or aureomycin were administered intramuscularly or intravenously, indicate that the mechanism by which antibiotics produce increased growth responses in higher animals is confined to the intestinal tract. Indeed, it is believed by some investigators that the effect is due to the action of the antibiotic upon the intestinal flora. Yet these responses are obtained in all instances with subinhibitory amounts of

[57] McGinnis, J., Stephenson, E. L., Levadie, B. T. H., Carver, J. S., Garibaldi, J. A., Ijichi, K., Snell, N. S. and Lewis, J. C. Response of chicks and turkey poults to Vitamin B_{12} supplements produced by fermentation with different organisms. Abstracts of Papers, 116 Meeting Amer. chem. Soc., 1949, 42A.

[58] Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C., Jr. and Franklin A. L. The multiple nature of the animal protein factor. *J. biol. Chem.*, 1949, *180*, 647–653.

[59] Stokstad, E. L. R. and Jukes, T. H. Further observations on the "Animal protein factor". *Proc. Soc. exp. Biol., N. Y.*, 1950, *73*, 523–528. Growth promoting effect of aureomycin on pigs. *Arch. Biochem.*, 1950, *26*, 324–325.

[60] Lueoke, R. W., McMillen, W. N. and Thorp, F., Jr. The effect of vitamin B_{12}, animal protein factor and streptomycin on the growth of young pigs. *Arch. Biochem.*, 1950, *26*, 326–327.

[61] Whitehall, A. R., Oleson, J. J. and Hutchings, B. L. Stimulating effect of aureomycin on the growth of chicks. *Proc. Soc. exp. Biol., N. Y.*, 1950, *74*, 11–13.

[62] Sieburth, J. McN., Gutierrez, J., McGinnis, M., Stern, J. R. and Schneider, B. H. Effect of antibiotics on intestinal microflora and on growth of turkeys and pigs. *Proc. Soc. exp. Biol., N. Y.*, 1951, *76*, 15–20.

[63] Reynolds, W. M., Weber, E. M. and Luther, H. G. Terramycin effect on growth of chicks. *Antibiotics and Chemotherapy (in press, July 1951).*

antibiotics only, that is, with concentrations below those necessary for inhibition of the organisms within the intestinal tract.

Nothing has been said, as yet, concerning the advances which have been made in the field of chemotherapy during the past decade although these may be considered among the most important and most extensive. It is of interest that the first experiments in this field were performed by two investigators who did most to establish bacteriology itself as a science. In 1877, Pasteur and Joubert[64] noted the antagonistic effect of aerobic bacteria on the growth of *B. anthracis,* and even observed that death of animals infected with anthrax sometimes could be prevented by including in the infecting dose of anthrax bacilli certain aerobic organisms. Pasteur ascribed this effect to the consumption of oxygen by the aerobic organisms, but Babes in 1885 suggested that the effect might be due to a chemical substance produced by the antagonistic organism. In 1881, Koch[65] infected guinea pigs with anthrax bacilli, and treated them with mercuric chloride administered subcutaneously. Mercuric chloride was chosen for this study because of its powerful *in vitro* bactericidal action. The animals died of anthrax within 48 hours despite the administration of dosages of mercuric chloride, both before and after infection, sufficient to prevent all bacterial growth *in vitro.*

The word "chemotherapy" was introduced by Paul Ehrlich in the early part of the twentieth century to denote the use of substances other than "antibodies" for the control of infection in animals and man.[66] To Ehrlich must go the credit for the initial development of the science of chemotherapy. Yet possibly due to the simultaneous development of the science of immunology, it was not until the advent of the sulfonamides in 1935 that attention was concentrated on evaluation of the potential significance of chemotherapeutic agents in the treatment of bacterial infections. The techniques developed during the study of this group of compounds served as a basis for subsequent investigations on penicillin, streptomycin, and newer substances. Only a portion of these techniques may be considered microbiological in nature, yet others of them bear direct relationship to the antimicrobial activities of the agent and hence must be considered.

The remarkable activity of the antibiotics, and of penicillin in particular, has accounted for the rapid development of these substances as chemo-

[64] Pasteur, L. and Joubert, J. F. Charbon et septicemie. *C. R. Acad. Sci., Paris,* 1877, *85,* 101–105.

[65] Work, T. S. and Work, E. *The basis of chemotherapy.* New York, 1948, p. 7.

[66] Ehrlich, P. *Das Sauerstoff-bedurfniss des Organismus; eine farbenanalytische Studie.* Berlin, 1885.

therapeutic agents, but likewise has been a handicap to their study. The growth of many strains of pathogenic pneumococci and streptococci is inhibited by as little as 0.006 to 0.012 mcg. of penicillin per ml., while many other pathogenic micro-organisms often are inhibited by less than 1 mcg. of this or other antimicrobial agents per ml. That serum may alter the degree of sensitivity of some organisms to certain of the antibiotics has been proven. One cannot question the fact that the successful use of a drug necessitates its reaching the site of action in sufficient amount and remaining there for a sufficient period of time to permit its action; if the action of a chemotherapeutic agent is directly on the parasite, its successful use must depend upon its reaching the parasite in a quantity in excess of that required to inhibit its growth.[67] With this in mind, it is evident that the determination of the optimum dosage, route, and frequency of administration of antimicrobial agents is dependent, at least in part, upon a knowledge of the amount of biologically active antibiotic in the tissues and fluids of the body. Only by accurate measurements can one be certain that a sufficient quantity of antibiotic is available at the necessary site for the period of time required for elimination of the infecting organisms. The development of bio-assay methods which would permit measurement of amounts as low as 0.03 unit or 0.018 mcg. of penicillin per ml., or 0.1 to 0.5 mcg. of other antibiotics per ml. of serum has been accomplished by modifications of the serial dilution assay, and the Oxford cup plate method mentioned previously. The methods most commonly used today are modifications of the original serial dilution technique introduced for determination of penicillin serum levels in 1940 to 1941.[68,69] These have been of inestimable importance and have aided materially in the evaluation of the chemotherapeutic action of antimicrobial agents and their dosages. As stated by Marshall,[67] "both the practical and theoretical aspects of chemotherapy have advanced more rapidly, because of our ability to determine blood and plasma concentrations of the antimicrobial agents, than would have been possible otherwise."

It is obvious from the information accumulated and from the techniques developed during the past ten years that the potentialities in the field of antimicrobial agents are great. The criteria for a successful chemotherapeutic agent have been well established during this period and may be restated as follows: (i) It must be highly active against pathogenic micro-organisms or parasites; (ii) it must be absorbed readily in the body; (iii) it

[67] Marshall, E. K., Jr. *The evaluation of chemotherapeutic agents.* New York, 1949, p. 4.

[68] Dawson, M. H., Hobby, G. L., Meyer, K. and Chaffee, E. Penicillin as a chemotherapeutic agent. *Ann. int. Med.*, 1943, *19*, 707–717.

[69] Dawson, M. H. and Hobby, G. L. The clinical use of penicillin. *J. Amer. med. Ass.*, 1944, *124*, 611–622.

must be active in the presence of tissue and other body fluids; (iv) it must possess a low degree of toxicity, and thereby a high chemotherapeutic index; (v) it should not permit the emergence of resistant cells. Methods are available for the isolation of large numbers of antimicrobial agents. Nonetheless, the evaluation of whether or not they fit the essential criteria established for such agents is more difficult, for, as already indicated, little is known concerning the mechanisms by which these agents exert their effect. Why do penicillin and bacitracin act primarily against gram-positive micro-organisms, while streptomycin exerts its antimicrobial effects against gram-negative organisms and the acid-fast bacilli? Why also are aureomycin, chloramphenicol, and terramycin effective against both of these groups of micro-organisms as well as others? These questions are among the most interesting ones confronting the bacteriologist, biochemist, and physician today.

Bacteria possess tremendous metabolic activities. Each species of bacteria requires certain specific vitamins, amino acids, and foodstuffs for growth and survival; indeed, their requirements may be as precise as those of man. It is recognized that any antimicrobial agent which acts only by virtue of its ability to lyse or coagulate the bacterial cell might have a similar effect upon tissue cells. It is suggested that certain of the effective antimicrobial agents may exert their effects by virtue of the ability to compete with one of the essential metabolites of the bacterial cell, and the evaluation of substances closely related to certain of the essential metabolites of specific bacteria eventually may provide one useful approach in the search for new antibiotics.

The possible use of two or more antimicrobial agents in combination with each other has been mentioned. In mixed infections due to more than one organism, the simultaneous use of combinations of antibiotics of differing antimicrobial spectra frequently results in complete sterilization, while use of one agent alone merely eliminates a portion of the organisms present, thereby leaving a fertile field for the propagation of the residual cells. The number of possible combinations is dependent only upon the number of antimicrobial agents available for use and upon their pharmacological action.

Nothing has been said concerning the many ways in which the host may modify a drug, thereby interfering with its action. Even today it too often is assumed that antimicrobial activity demonstrable *in vitro* will be apparent *in vivo* also; too often it is taken for granted that results obtained in one species of animal may be used as a basis for conclusions pertaining

to other species. The tendency remains, furthermore, to accept as proven statements to the effect that an antimicrobial agent is effective against one disease because it is effective against another caused by the same micro-organism. Sulfamerazine is a highly active antimicrobial agent *in vitro,* yet is acetylated *in vivo* to such an extreme degree that it is relatively ineffective; chloramphenicol is a most satisfactory chemotherapeutic agent for many infections in man, yet is essentially ineffective in mice; penicillin K is more active *in vitro* against certain micro-organisms than is the commonly used penicillin G, yet it is not used therapeutically due to the fact that it is bound to plasma proteins in humans to too great an extent. The effect of body fluids and, furthermore, individual and species differences cannot be ignored. Current studies on streptokinase, an enzyme produced by certain strains of hemolytic streptococci and capable of lysing fibrin clots, call attention to the possible effect of enzyme therapy on the chemotherapeutic action of antimicrobial agents. The introduction of ACTH (adrenocorticotropic hormone) and cortisone into the field of medicine brings up the question of the possible effect of agents in general which may alter host mechanisms. As stated recently by McDermott:[70] "The ultimate effectiveness of an antimicrobial drug in the treatment of a particular disease is conditioned by the pharmacologic properties of the drug, the efficiency of the host defenses and the speed with which they can be mobilized, and the nature of the lesions characteristic of the particular infection."

Those microbiological aspects of antimicrobial agents which pertain to their action against human pathogens have been considered primarily in this discussion. There is no question but that with present understanding of the antimicrobial properties of substances existing in the many bacteria, fungi, and higher plants which constantly surround us, and with modern chemical methods for isolating these in pure form, tremendous possibilities exist for the development of better chemotherapeutic agents. With our present knowledge of biochemical mechanisms and immune processes in the host, every opportunity exists for the development of more efficient forms of therapy. Of greater importance for future investigation, however, are the effects produced by interference with cell metabolism. One may wonder what may be the outcome of the use of substances produced by one micro-organism in order to suppress another, thus allowing a third to grow, and the use of subinhibitory amounts of these same substances to stimulate cellular growth. The question arises: What are we doing when we interfere with microbial civilization?

[70] McDermott, W. *The evaluation of chemotherapeutic agents.* New York, 1949, p. 92.

The Antibiotics and Pharmacy

GEORGE URDANG*

The Meaning and Purpose of "Pharmacy"

THE etymological derivation of "pharmacy" is well known. It stems from the Greek term *pharmacon* meaning in its historical development first a charm, then a poison as well, and finally a drug with emphasis on its use in therapy, i.e., as a health-restoring or sickness-preventing agent, without losing its relationship to charm and poison. Hence pharmacy represents the sum total of all activities devoted to the preparation and distribution of drugs. As an auxiliary to medicine it is, like medicine, not a so-called pure science based on and developing its particular laws which may or may not lead to practical results or uses, but the beneficiary of whatever the sciences may offer for application to its purposes.

In other words, whether it is chemical, physical, microbiological, or engineering research, the concepts and work which go into finding and developing new drugs and finally making their administration most adequate and their availability most convenient for the populace—this is known as pharmacy. It is pharmacy, represented by the manufacturing concerns, which is responsible for the important task of translating *in vitro* findings of research workers into large scale production and of meeting the requirements set by pharmacological and clinical observations. It is pharmacy, represented by scientists in the laboratories of industry, governmental agencies, and universities, which is working out assays, tests, methods of stabilization, and formulas for topical preparations—and it is remarkable that much of this work has been done in schools of pharmacy and in hospital pharmacies. Finally, it is pharmacy, represented by wholesale, hospital, and retail pharmacists, which guarantees the adequate distribution of the drugs needed, with the practitioners in hospital and retail pharmacies taking most adequate care of the small-scale preparation of drugs, thus meeting the medical requirements for individual administration.

In retracing the pharmaceutical history of the antibiotics in the first decade of their development from an interesting scientific discovery to the most important group of life-saving drugs ever known, this writer deliberately limits himself principally to publications in the representative American pharmaceutical journals. In his opinion, the mere fact that these journals have become the place for immediately recording and discussing

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every important step in the progress of manufacture, testing, and distribution of the products concerned, testifies to the sincerity with which American pharmacy in general has viewed its part in the development of antibiotics and their adequate use. It might be stated that although the references quoted herein have been limited mostly to the two editions of the *Journal of the American Pharmaceutical Association* (*Scientific Edition* and *Practical Pharmacy Edition*), the *American Professional Pharmacist,* and the *Bulletin of the American Society of Hospital Pharmacists,* there has been hardly one journal or magazine serving the drug trade in the United States that has not informed its readers of the development in the field of antibiotics. Most of these periodicals have rather early established special rubrics for references to pertinent publications and quotations therefrom.

The Beginnings of Large Scale Production

In the Kremers-Urdang *History of Pharmacy*[1] this writer made the following statement:

> Manufacturing pharmacy in America has been the child of wars. It was born during the Revolutionary War (1775–1783). It took the decisive step from childhood to manhood after and in consequence of the Civil War (1861–1865), and it became independent from Europe and dominant on the world market after World War I (1914–1918). World War II (1941–1945) made this domination a generally accepted fact.

However, there was a remarkable difference between the incentives motivating American pharmaceutical industry in former wars and the situation at the start of World War II. In the more than two decades after World War I, scientific research and its utilization by industry had developed in the United States to such an extent that its former dependence on the Old World for scientific concepts and technological skill had been almost reversed. Hence this time it was not replacement or imitation of European achievements that was required and more or less all that could be expected. Mature American industry was abreast of the time in independent research in all fields and projects promising practical results and it was able to put its own knowledge and experience to immediate use.

As far as antibiotics are concerned, W. H. Engels,[2] after having referred to the discovery in 1939 of tyrothricin, "an antibiotic that was announced

[1] Kremers, E., and Urdang, G. *History of pharmacy.* Philadelphia, J. B. Lippincott Co., 1951, p. 427.

[2] Engels, W. H. The industrial development of antibiotics. *Chem. Engng. News,* 1948, *26,* 1284.

by R. J. Dubos and collaborators at the Rockefeller Institute," stated the following:

> The research men of Merck & Co., Inc., interested themselves in Dubos' antibiotic, and, as the result of working on the production and purification of tyrothricin, acquired valuable experience in the technique of developing antibiotics which was put to good use when our attention was attracted by publications of Florey and his collaborators on penicillin. . . .
>
> In the meantime the war had started in Europe, which made a rapid development in England of the production of this promising drug very difficult. Although at Merck & Co., Inc., we had already started considerable work on penicillin, we agreed, urged on by Florey and by government authorities, to collaborate with others in the development of this product. . . . Practically all phases of the problem had to be tackled at once, and it should be said here that the rapid development of our penicillin process would not have been possible if we had not had the benefit of frequent consultations with others, especially the microbiologists, chemists, and chemical engineers of Charles Pfizer & Co., of E. R. Squibb & Sons Co., and of the Northern Regional Research Laboratory.

The early development of large scale penicillin production in England, briefly referred to by Engels, is described by Perlman[3] as follows:

> This need was first recognized in Great Britain and resulted in the formation of the Therapeutic Research Corporation whose membership included representatives of the Boots Pure Drug Company, Ltd., British Drug Houses, Ltd., Glaxo Laboratories, Ltd., May and Baker, Ltd., and the Wellcome Laboratories. Research progress reports were circulated among these members and in 1942 the importance of the work caused the Ministry of Supply to organize a general commission to deal with the various aspects of the production of penicillin—the Penicillin Producers Conference. This group included at times members of the above group and representatives of Imperial Chemical Industries, Ltd. (Alkali and Pharmaceutical Divisions), Imperial College of Science, Manchester University, the National Institute for Medical Research, and several laboratories of Oxford University.[4]

There was a continuous exchange of information between British and American laboratories. As to the chemistry of penicillin "over 700 reports were exchanged in less than three years."[5]

The way in which early co-operation in the United States between government agencies, pharmaceutical industry, and academic scientific research was established and carried through has been described in greater detail by the pharmacist, Fred J. Stock (then head of the Drugs and Cosmetics Branch, Chemicals Bureau, War Production Board) in an article on penicillin production and distribution published in a special issue (April

[3] Perlman, D. Some mycological aspects of penicillin production. *Bot. Rev.*, 1950, *16*, 449–523. This special issue (No. 9, November) represents an authoritative survey of the subject. Having taken part in the co-operative research project on mycological penicillin at the University of Wisconsin, and with later experience in the manufacture thereof, Perlman has a rather rare insight, and his arrangement of the subject matter is excellent. His bibliography (275 references) offers a wealth of information.

[4] *Ibid.*, 454.

[5] *Ibid.*, 455.

1945) on penicillin of the *Journal of the American Pharmaceutical Association, Practical Pharmacy Edition.*[6] Of his statements the following are of particular interest:

> . . . More than 175 companies were interviewed or corresponded with [by the War Production Board] regarding penicillin production. Twenty-one companies were selected on the basis of their experience with penicillin, their knowledge of the production of chemicals by the fermentation method, their general experience with biological products or the processing of blood plasma and serum albumin, the technical staff, facilities available and other pertinent factors. . . . Invaluable technical assistance was given by the office of Production Research and Development which also initiated a number of research projects at Pennsylvania State College [extraction and purification processes], University of Wisconsin [fermentation process and growth requirements of the mold], Massachusetts Institute of Technology [drying of the final product], University of Minnesota, and Stanford University [genetics of the penicillium mold] to assist in the solution of the technical problems incident to the transfer of production from laboratory to commercial plant scale.

These academic researches furnished excellent results, supplementing those obtained in the industrial laboratories. The translation of all the experimental work into large scale production was naturally the exclusive task of the pharmaceutical industry.

The following manufacturers are named by the editor of the April 1945 issue of the *Journal of the American Pharmaceutical Association, Practical Pharmacy Edition,* in a "latest available list of pharmaceutical and chemical laboratories [in the U.S.A.] engaged in the manufacture of penicillin": Abbott Laboratories; Ben Venue Laboratories, Inc.; Cheplin Laboratories, Inc.; Commercial Solvents Corporation;[7] Cutter Laboratories, Inc.; Heyden Chemical Corporation; Hoffman-La Roche, Inc.; Lederle Laboratories, Inc.; Eli Lilly and Company; McKesson and Robbins, Inc.; Merck and Company, Inc.; Parke, Davis and Company; Chas. Pfizer and Company, Inc.; Schenley Laboratories, Inc.; E. R. Squibb and Sons; The Upjohn Company; Winthrop Chemical Company, Inc.; Wyeth Incorporated.

The Development of the Technical Processes

Of the research conducted by members of academic staffs, that pursued

[6] 1945, *6*, 89–132. The other essays in this special issue came from the Food and Drug Administration (H. Welch, R. P. Herwick, A. C. Hunter: Properties and Standardization), the Mayo Clinic (D. H. Heilman, W. E. Herrell: Biologic Characteristics), the Chas. Pfizer Company (Scientific and Engineering Staff: Present-day Production Methods), Yale University School of Medicine (John S. Lockwood: Considerations in Therapy), E. R. Squibb and Sons (R. J. Dahl: Pharmacy and Formulation). This special issue was published under the editorship of Glenn Sonnedecker, now Secretary of the American Institute of the History of Pharmacy, University of Wisconsin School of Pharmacy, Madison, Wisconsin. Mr. Fred J. Stock now holds the position of Vice-President of the Chas. Pfizer Company.

[7] The Commercial Solvents Corporation issue a periodical devoted exclusively to information on antibiotics.

at the University of Wisconsin proved to be of particular importance. The editor of *American Professional Pharmacist* published an account of the Wisconsin achievements (and about the availability of "crystalline, reaction free penicillin, not requiring refrigeration") in the April 1946 issue of his journal under the sensational heading "Penicillin 'blows up'." The note on the Wisconsin findings reads in its essential part as follows:

> Research workers at the University of Wisconsin have developed a new strain of penicillin which has opened the possibility of doubling and tripling the nation's supply of penicillin. The feat was accomplished by exposing the spores of the penicillin-producing mold to powerful ultraviolet rays. . . . This strain, known as Q176, is not patented and is therefore available to all penicillin manufacturers.[8]

It is, indeed, this strain (grown on media supplemented with phenylacetyl type compounds) which is still used by most manufacturers. The yields have been increased, furthermore, by employing a dilute suspension of corn steep liquor as the basis of the culture medium in which the mold is produced.[9] Finally, the change from "surface culture" to "submerged culture" techniques has, according to estimates reported by Pratt and Dufrenoy[10] "increased the amount of penicillin produced 20-fold in terms of space required for biosynthesis, and 72-fold in terms of man hours. Total industrial output was further increased by more and larger fermentation tanks . . ."

The development of antibiotic fermentation, filtration of antibiotic broth, and of penicillin extraction has been described by Colin and Silcox[11] as follows:

> *Antibiotic Fermentation.* The first penicillin made available for use was produced by surface fermentation in glass bottles. These bottles were capable of yielding approximately 0.5L. of broth after several days of fermentation. Even in small scale operation, the number of bottles to be handled made the process unwieldy. It was necessary at that time to carry out this fermentation under conditions of shallow liquid depths since the fermentation was aerobic. The first step toward large scale production was to

[8] *Amer. prof. Pharmacist,* 1946, *12,* 335.

[9] Discovered by Moyer and Coghill of the Northern Regional Research Laboratory of the U. S. Department of Agriculture. See also: Liggett, R. W., and Koffler, H. Corn steep liquor in microbiology. *Bact. Rev.,* 1948, *12,* 297–311. Dr. Koffler took part in the co-operative project at the University of Wisconsin mentioned above and directed by Drs. M. P. Backus, R. H. Burris, M. J. Johnson, E. McCoy, W. H. Peterson, and J. F. Stauffer.

[10] Pratt, R., and Dufrenoy, J. *Antibiotics.* Philadelphia, J. B. Lippincott Co., 1949, p. 33. The authors' estimate is based on data furnished them by the Cutter Laboratories, Berkeley, California.

[11] Colin, P. G., and Silcox, H. E. The chemical engineer. *J. Amer. pharm. Ass., Pract. Pharm. Ed.,* 1947, 8, 137–141. Both authors base their reports on experience gained as members of the staff of Merck & Co., Dr. Colin as Director of Chemical Technology, Dr. Silcox as Head of Chemical Engineering and Development. The article which appeared in a special issue of the Association Journal (March 1947) entitled "Birth of a new drug," described the part taken by representatives of the various sciences and professions in the development of new drugs. The issue was edited by Glenn Sonnedecker (see note 6).

carry out the fermentation in stacks of shallow trays placed in a steel shell. Before these units were put in operation, however, microbiologists developed a mold strain capable of submerged production of penicillin which was adaptable to tank fermentation, provided air was introduced through spargers into the fermenting batch. Based on this development, the steel shells for holding the shallow trays were converted to deep tank fermenters. Since that time these deep fermenters have been modified to incorporate agitation, improved methods of air dispersion and many other features advantageous for antibiotic fermentation. In the short space of five years [1942–47], the original bottle fermentation has evolved to the 15,000 gallon fermenters which are used for the fermentation of streptomycin.

Filtration of Antibiotic Broths. . . . Several years ago when antibiotic production was first contemplated, the filter pot[12] was used for broth filtration of the initial small production. As production increased, it became apparent that such a filtration mechanism would not only be impractical but also impossible. The next unit tried was the centrifuge, but because of broth characteristics this method soon gave way to the plate and frame filter press. As antibiotic production pyramided, it soon became apparent that several hundred thousand gallons per month of these comparatively unstable broths, containing mycelia and slimes, would have to be filtered at prescribed temperatures within short time limits. After considerable experimentation, the continuous rotary filter was chosen.

In production, rates of several thousand gallons per hour have been maintained using rotary pressure precoat units with advancing knives and spray washing. The continuous presentation of fresh filtering surface by the precoat filter has made it possible to attain high filtration rates and high antibiotic recovery, and the use of this filter has been one of the important contributions to large-scale antibiotic production.

Countercurrent Extraction of Penicillin. . . . Solvent extraction is used considerably as part of the penicillin isolation procedure. At the outset these processes were based on cross extraction. . . . [Later] special units were assembled that allow rapid mixing and centrifugal separation on a countercurrent basis. These effect efficient transfer of penicillin into and out of organic solvents.

When penicillin was first made available, the potency (and thus the purity) of the final product was low. This was due to the presence of impurities. . . . By quantitative evaluation of the extraction properties of the impurities and the determination of the slight differences in distribution coefficients, it has been possible to extract penicillin selectively by the imposition of very closely controlled extraction conditions. Although it has not been possible to produce pure penicillin by extraction alone, these improvements in extraction techniques have contributed much toward making a high purity product available.

That the top level of potency was reached in preparations showing an activity of 1600 Oxford units per milligram was verified "when Dr. Wintersteiner of Squibb succeeded in obtaining the sodium penicillin in crystalline form."[13] At about the same time, collaborating British scientists had likewise isolated pure penicillin (1945–46). "Commercial penicillin now

[12] The "filter pot" is the counterpart in production, still used for some small-scale application, of the "Büchner funnel," customarily employed for filtration in the organic laboratory.

[13] Engels, *op. cit.* This important deed was achieved by Wintersteiner and MacPhillamy of the Division of Organic Chemistry of the Squibb Institute for Medical Research in July 1943. Cf. Coghill, R. D., *Chem. Engng. News*, 1944, *22*, 588.

available," stated the Council on Pharmacy and Chemistry of the American Medical Association in the middle of 1946, "is predominantly penicillin G. . . ."

The particular difficulties to be overcome in the industrial development of streptomycin have been intimated in the article by W. H. Engels referred to above. The following excerpts give testimony to the courage and resourcefulness of American industry in the development of a drug that offered great promise but could nevertheless have been a failure:

> As in the case of penicillin, our [Merck & Co's] research men had to tackle all phases of the program almost simultaneously. . . . Pilot plant production in deep fermentation tanks, available from another process, began immediately [in 1944] and we were soon able to produce sufficient quantities of the crude drug to enable our organic chemists to develop purification methods and to elucidate the structure. At the same time, an intensive study of the pharmacology and efficacy in animals was started at the Merck Institute and considerable quantities of material were made available to outside collaborators, especially for testing its effectiveness against tuberculosis infections in animals. . . .
>
> As soon as the first encouraging clinical reports were made available, the process was frozen—i.e., no further deviations from the then-established process were permitted for the production intended for clinical investigation, unless each variation was evaluated not only by the microbiological assay, by toxicity and efficacy determinations in animals, and by chemical and physical standards, but also by clinical behavior of the resulting product. . . .
>
> The management's decision to go ahead with erection of a large scale plant put, therefore, a tremendous pressure on our [Merck & Co's] development men. But, as is so often the case, necessity is the mother of invention, and our chemists and chemical engineers succeeded in developing an extraction and purification process which depended on a principle not often before used in the manufacture of chemicals on a large scale and which had no resemblance to the method we had been using heretofore. . . . In spring of 1946, streptomycin hydrochloride of the desired quality was being produced in excess of design capacity. . . .

As to the development of a reliable chemical assay replacing the "tedious and not too reliable microbiological" one, Dr. Engels states that already at an early stage "Dr. Boxer of our laboratories succeeded in developing one depending on the formation of maltol which had been reported by research workers at the Abbott Laboratories (Schenck and Spielman) as an alkaline degration product of streptomycin." This chemical assay is now applied "even to fermentation broths."[14]

It is of interest that in 1943, when the production of antibiotics was still in its infancy, uncontrolled and hardly available, a "kitchen method of producing penicillin gauze" was advocated, "proposed to the Industrial

[14] For information about present-day industrial production and control of antibiotics, see Pratt and Dufrenoy, *op. cit.*, pp. 27–54, and p. 126 for an account of extraction and purification of streptomycin.

Hygiene Foundation by Dr. Julius A. Vogel, plant physician for the Jones and Laughlin Steel Corporation." The heading of the respective pronouncement in the *Journal of the American Pharmaceutical Association, Practical Pharmacy Edition*[15] read: "Pharmacists can produce penicillin." This statement was very soon counteracted by an emphatic warning against dangers in "home-made" penicillin published in the same journal and the editor of the *American Professional Pharmacist,* Dr. John N. McDonnell (chosen as Director of the Office of Civilian Penicillin Distribution of the War Production Board in 1944, now Vice-President of the Schering Corporation, Bloomfield, N.J.), warned in the January 1944 issue of his journal against "home-made" penicillin as not only dangerous but also unnecessary. Since penicillin had been made available for general (although still channelled) use late in 1943 and, as McDonnell put it, "new plants now are daily supplying increasing quantities of the material," any attempt at home production of penicillin had become unnecessary indeed. Besides, it was simultaneous with the availability of penicillin for general use that the Food and Drug Administration was charged with the responsibility of supervising its production. Later acts of Congress extended this responsibility first to streptomycin and then to all other antibiotics. The present-day situation is described by Pratt and Dufrenoy[16] as follows:

> Detailed specifications for different antibiotics and prescribed methods for conducted tests are published by the Food and Drug Administration, a division of the Federal Security Agency.
>
> Rigid control is exercised over all antibiotics. Each manufacturer conducts the required tests on each lot of his own product and also sends samples of each batch to the F.D.A. laboratories where similar tests are performed. If the product passes all tests in both laboratories, the F.D.A. certifies the product for release to the pharmacist. Then, and not until then, can the manufacturer legally distribute the product.

It was through this continuous control that the growth of antibiotics production was kept within the limits required by public health considerations and that the numerous new products following their prominent sisters, penicillin and streptomycin, and admitted by the F.D.A., were the result of close scrutiny. It is furthermore apparent that the F.D.A. laboratories, in the course of controlling, became of necessity themselves places of fruitful research, thus contributing to the progress in the field. And finally, being a branch of a democratic government, the F.D.A. could not limit itself to supervision, the setting of rules, and the prosecution of violators, but had to meet another and very urgent requirement—that of information. It must be stated, with much appreciation and gratitude, that the F.D.A. has

[15] 1943, *4*, 342.

[16] *Op. cit.*, p. 53.

fulfilled all the tasks listed above, including the rather difficult one of informing the interested groups as quickly and completely as possible.

The Spread of Information to and through Pharmacy

In the special April 1945 issue of the *Journal of the American Pharmaceutical Association, Practical Pharmacy Edition,* to which reference has several times been made, the editor, Glenn Sonnedecker, had expressed the hope "that this issue will serve as a reference handbook and guide through which pharmacists may dispense penicillin wisely and discuss intelligently with their physicians the questions that will arise . . .," and the Secretary of this representative national organization of American pharmacists, Dr. Robert P. Fischelis, wrote the following:

> Just as the physician must be definitely sure of the proper application of penicillin in the specific case for which he is prescribing, so the pharmacist must be sure of his procedure in dispensing this drug. This involves a knowledge of the keeping qualities of the drug, the extent to which its incorporation in certain vehicles or bases may inhibit its action, the keeping quality of the finished product and its consequent effectiveness at the time of use, and the possibility of reactions by the sensitive or sensitized patient.

This not only states clearly and profoundly the tasks to be met by the dispensing (retail and hospital) pharmacists, but intimates likewise the direction in which research may be of special interest for these groups, particularly for the hospital pharmacists. Above all, continuous information was needed.

As stated in the introductory remarks, this duty was recognized and met by the entire pharmaceutical press, but was especially well handled by the leading journals in the professional-scientific field: the *Journal of the American Pharmaceutical Association* (*Scientific and Practical Pharmacy Editions* being the contact organs of organized pharmacy in governmental and academic circles), the *American Professional Pharmacist* (specializing in informative surveys), and the *Bulletin of the American Society of Hospital Pharmacists,* which was started in 1945 and has steadily gained in volume and importance. It was the *Journal of the American Pharmaceutical Association* in which the reports on original scientific research thought to be of pharmaceutical interest appeared primarily and which was the preferred place of publication for work done in the laboratories of the Food and Drug Administration.

It is significant of the semi-official nature of the *Journal of the American Pharmaceutical Association* that it did not pay much attention to the

new group of drugs up to the end of 1944, i.e., until that matter had obviously become one of official concern. There was in the *Pharmaceutical Abstracts* (a supplement to the aforementioned *Journal*) one reference to penicillin as early as 1941 and another in 1942, increasing in 1943 to twenty and in 1944 to not less than fifty references. The *Practical Pharmacy Edition* of the *Journal* published its first note on penicillin in October 1943, followed in November by a report on a "home-made" product mentioned above, and added nine notes in 1944. But the *Scientific Edition* of the *Journal* remained closed to the new drugs until early in 1945 when it published its first paper on an antibiotic—a comprehensive survey that came from the Food and Drug Administration. Its title was "Penicillin—a Review."[17] In the same year there appeared two more articles by members of the scientific staff of the F.D.A., and an article by Selman A. Waksman and Albert Schatz on "Streptomycin—Origin, Nature, and Properties."[18] An abbreviated reprint of the latter article was published in the *Practical Pharmacy Edition*[19] as a kind of supplement to the special April 1945 antibiotic issue referred to before.

From then on, a veritable flow of original reports have appeared in the *Scientific Edition,* and of surveys, notes, and references in the *Practical Pharmacy Edition* of the *Journal.*

Of the eight articles published in 1946 in the *Scientific Edition* (one on antibiotics in general, one on tyrothricin, and six on penicillin or penicillin products), two came from the laboratories of the F.D.A., three from industrial concerns, three from academic institutions. One of the latter originated in the Purdue University School of Pharmacy. In 1947 it published ten articles on penicillin and one on streptomycin. Of these eleven papers, not less than seven came from the laboratories of the F.D.A., two from industrial concerns, and two from the University of California College of Pharmacy. In 1948 the same *Journal* presented nine original articles on penicillin. Again the Food and Drug Administration was the main contributor, four of the articles originating from its scientific staff members, while two came from the laboratories of industrial concerns and three from the University of California College of Pharmacy which, due mainly to the work of Robertson Pratt and Jean Dufrenoy,[20] had become an important contributor to antibiotic research. In 1949, two of the eight articles on anti-

[17] *J. Amer. pharm. Ass., Sci. Ed.*, 1945, *34*, 97–109.

[18] *Ibid.*, 273–291.

[19] *J. Amer. pharm. Ass., Pract. Pharm. Ed.*, 1945, *6*, 308–321.

[20] See note 10. It is of interest that the research on antibiotics conducted at the University of California College of Pharmacy has been generously supported by the Cutter Laboratories, Berkeley, California.

biotics published in the *Scientific Edition* of the *Journal of the American Pharmaceutical Association* reported on research done by staff members of the Food and Drug Administration, three came from industry and three from Colleges of Pharmacy (two from the University of California and one from the University of Minnesota). In 1950, finally, three of the nine respective articles published in this representative organ of American pharmacy came from industry, one from a college of pharmacy (University of California), and the majority, five, once again from the Food and Drug Administration.

The reason for the temporary decline in contributions from the Food and Drug Administration in 1949 and its renewed upsurge in 1950 becomes obvious from the particular topics dealt with in the respective publications. Up to 1948 it was mainly penicillin and its products which had been the subject of investigation and information by the Food and Drug Administration. In 1950 not one of the nine articles on antibiotics published in the *Journal of the American Pharmaceutical Association, Scientific Edition* dealt with penicillin. Instead, it was aureomycin, bacitracin, streptomycin, terramycin, and tyrothricin which now demanded and were given attention and critical evaluation, the results of which were presented by the Food and Drug Administration.

This development is mirrored likewise in the *American Professional Pharmacist* and the *Bulletin of the American Society of Hospital Pharmacists.* Penicillin did not lose its importance but had to yield publicity value as well as necessity to the more recent antibiotics. A scanning of the indices of the *American Professional Pharmacist* for references (articles, notes, etc.) to the various antibiotics from 1945–1950 reveals the following: In 1945, 135 to penicillin, 5 to antibiotics in general, 1 to bactratycin, 4 to gramicidin, 13 to streptomycin, and 3 to streptothricin; in 1946, 89 to penicillin, 2 to antibiotics in general, 16 to streptomycin; in 1947, 60 to penicillin, 1 to antibiotics in general, 1 to bacitracin, 1 to chloromycetin (chloramphenicol), 16 to streptomycin, 2 to tyrothricin; in 1948, 40 to penicillin, 2 to antibiotics in general, 1 to aureomycin, 2 to bacillosporin, 2 to bacitracin, 1 to chloromycetin, 15 to streptomycin; in 1949, 14 to penicillin, 5 to antibiotics in general, 13 to aureomycin, 5 to bacitracin, 8 to chloromycetin, 2 to dihydrostreptomycin, 1 to neomycin, 12 to streptomycin; in 1950, 11 to penicillin, 4 to antibiotics in general, 12 to aureomycin, 5 to bacitracin, 3 to chloromycetin, 8 to dihydrostreptomycin, 2 to hydroxystreptomycin, 1 to lupulon, 3 to polymyxin, 14 to streptomycin, 3 to terramycin. The *Bulletin of the American Society of Hospital Pharmacists* adds references to aero-

sporin (1948), circulin and antibioticX-G (1949), luteomycin, and viomycin (1950).

The most important source of information to pharmacy (because it is binding) is naturally the pharmacopeia. Being an authoritative book, it mirrors in its additions as well as in its omissions the temporary stage of acceptance of the drugs concerned. It was not until 1944 that the United States Pharmacopœial Convention, Inc., which is responsible for the *United States Pharmacopœia* (since its fourteenth revision—1950—changed to "Pharmocopeia"), took into consideration the admission of antibiotics into the next (thirteenth) revision. As was to be expected, it limited its sanction to penicillin and penicillin products. The U.S.P. XIII, "official from April 1, 1947," contains sections on penicillin calcium, penicillin sodium, penicillin dental cones, penicillin injection in oil and wax, penicillin ointment, penicillin tablets, and penicillin troches. When three years later, "official from November 1, 1950," the U.S.P. XIV appeared, there were a few omissions (penicillin calcium, penicillin dental cones, penicillin injection in oil and wax) and a number of new additions opening the door for antibiotics other than penicillin although the latter still is the favorite: aureomycin hydrochloride, aureomycin hydrochloride capsules, chloramphenicol, chloramphenicol capsules, dihydrostreptomycin, penicillin potassium, penicillin G. potassium, penicillin G. procaine, penicillin G. sodium, buffered crystalline penicillin, penicillin for inhalation, penicillin procaine for aqueous injection, penicillin procaine in oil injection, streptomycin, tyrothricin, tyrothricin solution, tyrothricin spray, tyrothricin troches.

It is of interest that (with the exception of penicillin G. potassium, penicillin G. sodium, tyrothricin and its topical products listed above) for the antibiotics and their preparations few if any tests for identification, purity, etc. are given which warrant the genuineness and adequacy of the other drugs listed in the pharmacopeia. Instead, the respective sections carry the statement that "the requirements of the Federal Food and Drug Administration" are complied with. This naturally refers to the fact that the responsibility for the adequacy of these products rests with the F.D.A.

Topical Preparations

It is the field of topical preparations—their manufacture for immediate use primarily but also for later sale—which has long been the mainstay of the pharmaceutical practitioner after large-scale industry, developing mostly from the pharmaceutical retail store in the late eighteenth and the nineteenth centuries, had taken over the manufacture of the chemical (and

later on biological) substances requiring an amount and kind of planning and equipment far beyond the level of the pharmaceutical retail business. Gradually large-scale production took over the field of the topical preparations as well. However, there still has remained for the pharmaceutical practitioner the task of meeting individual prescription requirements as a small-scale manufacturer and, if working in a hospital pharmacy, even on a somewhat larger scale. It is there, in the working out and testing of solution and stability problems, in the selection and preparation of adequate ointment-bases, etc., that contributions by pharmaceutical practitioners have been offered.

After the distribution of penicillin had been freed from its initial restrictions in 1944, the Chief Pharmacist of the Buffalo General Hospital, F. X. Sturmer, published an article on "Compounding and Dispensing Techniques for Penicillin Products" in which, on the basis of his own experience, he discussed the preparation of penicillin solutions, wet dressings, ear drops, eye drops, and ointments.[21] His introductory remarks read as follows:

> The restrictions and system of distribution of penicillin during the past has enabled the hospital pharmacy to do work on penicillin products in a pioneering manner. This opportunity has opened a field for the development of new and distinct types of medication, and the experience and observations of the hospital pharmacist should be of immeasurable value to the retail pharmacist now that penicillin is available for his use.

At about the same time, this favorable opinion on the part of pharmaceutical practitioners in this field was confirmed by R. J. Dahl, a member of the staff of the great pharmaceutical manufacturing concern of E. R. Squibb and Sons, in an article published in the *Journal of the American Pharmaceutical Association*.[22] In presenting "the latest [1945] data on pharmaceutical forms" of antibiotics, Dahl makes "grateful acknowledgment . . . to the many pharmacists [who have helped him] by their splendid suggestions." Of the five selected formulas for penicillin ointments which Dahl presents, two were furnished by hospital pharmacists and one by a teacher in a pharmacy school. His standard formula for the preparation of penicillin pastilles is taken from a publication in the organ of the Pharmaceutical Society of Great Britain, *The Pharmaceutical Journal*.

Considering "injection forms" in ampoules as a class by themselves, Dahl listed as "topical forms" solutions, ointments, pastilles, powders, and

[21] *Bull. Amer. Soc. Hosp. Pharm.*, 1945, *2*, 74–76.

[22] Dahl, R. J. Penicillin pharmacy and formulation. *J. Amer. pharm. Assoc., Pract. Pharm. Ed.*, 1945, *6*, 120–129.

suppositories. Still in 1945 it was officially stated that "penicillin is not in sufficient supply to permit its use in manufactured products, such as pills, lozenges and other dosage forms."[23] One year later there was sufficient supply of penicillin and, as was to be expected, these "manufactured products" were immediately seized upon by large-scale manufacturing concerns. Since the commercial success of such prescription drugs depends on their acceptance by the medical profession, most U.S. manufacturers, after having obtained the sanction of the Food and Drug Administration, are eager to submit their products to the Council on Pharmacy and Chemistry of the American Medical Association for acceptance and admission to the Council's yearbook, *New and Non-Official Remedies*.

The Council accepted in 1946 the topical penicillin ointment as submitted by three concerns in addition to the non-topical penicillin calcium and sodium salts in vials (submitted by nine concerns) and penicillin calcium in oil and wax. In 1947 nineteen penicillin and seven streptomycin topical products were submitted by eleven concerns. In 1948 the number of A.M.A.-approved topical antibiotic preparations produced by large-scale industry had reached its height. There were listed by the A.M.A. Council on Pharmacy and Chemistry not less than thirty-five penicillin and seven streptomycin topical preparations offered by fifteen different concerns and comprising a variety of modes of application from solutions to troches, tablets, and ointments.

There was sudden stop of accreditation of topical antibiotic preparations by the Council in 1949, especially as far as penicillin products were concerned. Only certain topical forms of aureomycin were listed as accepted. The same holds true for 1950. Chloramphenicol (chloromycetin) is listed as a new drug, and chloramphenicol capsules are admitted as the only topical form of an antibiotic acknowledged by the Council in 1950. The reason for this attitude has been laid down in the report of the meeting the Council held on November 21–22, 1949 as follows:

> Because of the increasing instances of sensitization to penicillin preparations for local application, the Council voted that penicillin preparations marketed as liquids, ointments, and ophthalmic ointments be considered unacceptable for inclusion in N.N.R. [*New and Non-Official Remedies*] and that there be inserted in the appropriate N.N.R. section a warning concerning the danger of sensitivity following the topical application of penicillin products. . . .[24]

Stock in his article quoted above[25] had pointed to the fact that "marketing of these pharmaceuticals will have to await . . . (besides product

[23] Stock, F. J. Penicillin, production and distribution. *Ibid.*, 114.

[24] *Ibid.*, 1950, *11*, 174.

[25] *Op. cit.*, p. 114.

development) further clinical research." Apparently it was the accumulated experience of the medical practitioners which offered the facts on which the above-quoted decision of the A.M.A. Council is based. This decision naturally does not mean that the physician is not permitted (or will not) prescribe such topical penicillin preparations whenever he thinks it advisable in an individual case. It is for such cases that the pharmaceutical practitioner has to be prepared in order to meet his professional responsibilities towards society. To quote once again from the article by Dahl referred to above:

> The [practising retail or hospital] pharmacist is competent not only to be entrusted with the intelligent preparation of pharmaceutical forms, but also to be entrusted with an equally great concomitant responsibility—the responsibility for doing all in his power to prevent the misuse of a drug which, although the most powerful antibacterial substance known, is still not a 'cure all.'[26]

If one were looking for an example of the fact that large-scale manufacturing of drugs has not diminished the responsibilities of the well-educated pharmaceutical practitioner towards, and his necessity for, society, it is given here. There are limitations to both groups, and there is a mutual interdependence which cannot be neglected without harming society.

Although most of the pharmaceutical research has been centered in the laboratories of the large-scale manufacturers, the accumulation of experience in retail and hospital pharmacy has become obvious and fruitful in quite a number of publications designed to aid the pharmaceutical practitioner in his actual work with regard to antibiotic preparations. Some of these studies have been the result of practical experience and academic experimentation. One of the latest respective publications is by Herbert Flack (Chief Pharmacist, Jefferson Medical College Hospital, Philadelphia, Pennsylvania) and James Inashima (graduate student, Philadelphia College of Pharmacy and Science).[27]

Influence on the Business of Pharmacy

Manufacturing. One of the leading men in the American pharmaceutical industry, Mr. John E. McKeen (President of Chas. Pfizer & Co., Inc.), has on several occasions in 1950 pictured the changes in the production and distribution of drugs brought about by the development of antibiotics. In an address presented by him in March 1950 to the New York Society of Security Analysts he remarked that "penicillin and streptomycin have

[26] *Op. cit.*, p. 129.

[27] Inashima, J., and Flack, H. Pharmaceutical aspects of penicillin. *Bull. Amer. Soc. Hosp. Pharm.*, 1949, *6*, 102–107.

changed from high-margin pharmaceuticals to mass-produced, low-profit pharmaceutical staples. . . . At the present time approximately 45 per cent of Pfizer's total sales are in antibiotics. . . ."[28]

Mr. McKeen supplemented this statement in a talk to the Chicago Investment Analysts' Association by telling that "antibiotics now represent by all odds the largest single item in the entire ethical[29] line, exceeding in value even such items as ethical vitamins. In 1949, antibiotics accounted for 22 per cent of the total value of all ethical drugs manufactured and in 1948 the New York Department of Hospitals spent nearly one half of its total budget on penicillin and streptomycin alone."[30]

And finally, in May 1950, Mr. McKeen, in addressing the American Institute of Chemists, Inc., said the following:

> It is in fact only nine years since the first patient was unsuccessfully treated with penicillin in England. He died because the supply of penicillin was exhausted before he could make a complete recovery. . . . [Today], it is roughly estimated that antibiotics can be used with fair to excellent results in 30 to 50 per cent of the cases requiring the attention of the physician. These advances have given rise to the optimistic expectation that new antibiotics will be discovered which will be utilized in the treatment of presently uncontrolled diseases.[31]

There are official figures on the production of penicillin in the United States summarized in *Chemicals and Drugs,* a monthly publication of the U.S. Department of Commerce.[32]

Year	*Billion units*
1943	21
1944	1,633
1945	7,052
1946	27,809
1947	41,426
1948	95,855
1949	133,229

"Sales of penicillin products had an estimated value of $200,000,000 in 1948 and during the past six years the price of 100,000 units of penicillin has dropped from $20 (1943) to less than $0.10 (1949) with the purity of the product distributed increasing from about 25 p.c. to 98 p.c."[33]

This development in mass production and price reduction was natu-

[28] *Drug and Cosmetic Industry,* 1950, *66,* 393.

[29] "Ethical" in this connection means products sold by appeal to the medical and pharmaceutical profession rather than by direct appeal to the public.

[30] *Drug and Cosmetic Industry,* 1950, *66,* 645.

[31] *Ibid.,* 652.

[32] Quoted from Perlman, *op. cit.,* p. 457.

[33] *Ibid.,* 458.

rally not restricted to the United States. As to the situation all over the world with regard to penicillin D. Perlman furnishes the following data:

> . . . Considerable effort was expended in other countries, notably Great Britain. By the end of 1945 production in Great Britain was approaching 200 billion units per month; in Australia, 20 billion units per month; and small amounts were produced in Mexico . . . [After the end of World War II] production plants were started in nearly all of the European countries . . . making use of the processes developed in the United States and in many cases following laboratory studies concluded under wartime conditions. There are plants in 25 countries manufacturing penicillin, with nine in Great Britain, five in Germany, three in Italy and large installations in the Netherlands, Denmark and Sweden. Approximately 25 p.c. of the penicillin produced in the United States during 1949 was exported (value $40,000,000); this market is rapidly being reduced as the foreign plants reach maximum production.

Perlman refers furthermore to "a rather sizable penicillin industry" developed since the end of 1945 in Japan, to "some small plants . . . started in China during the war, based on processes used in the United States," and to the lack of information concerning penicillin production in Russia.

Retail. The statements made by the President of Chas. Pfizer & Co. Inc., (Mr. McKeen) as to the development of antibiotics research and production and its meaning to the manufacturing industry have been supplemented by Mr. Thomas J. Winn, sales manager of the same concern, with regard to retail pharmacy in the United States in an address before the Greater New York Drug Show in September 1950.[34]

> All of us [said Mr. Winn] can easily visualize the bigness of the so-called oral hygiene products' market—the tooth pastes, tooth powders, liquid dentifrices (etc.) . . .—add all of these products together and you have slightly less than half the estimated normal 1950 market for antibiotics, and antibiotics are dispensed only on prescriptions. Add together *all* the vitamin sales to all outlets (except hospitals) and it is not anywhere near as much as the 1950 sales of antibiotics will be. Add together all sales of hormones, botanicals and sulfonamide drugs—antibiotic sales are larger. . . . Antibiotics, it is estimated by market research people, will account for nearly $1.00 out of every $4.00 spent for prescription drugs this year. . . .[35]

The influence exerted within the frame of this development by and on the pharmaceutical practitioner has been characterized by Mr. Winn as follows:

> We think the wide dissemination of knowledge of these new products and the willingness of pharmacists, particularly, quickly to gain a working knowledge of them, better to counsel and advise members of other professions, physicians and nurses, has had much to do with the speed with which these new discoveries have been able to benefit patients.[36]

[34] *Amer. prof. Pharmacist*, 1950, *16*, 1981–1986.

[35] *Ibid.*, 985.

[36] *Ibid.*, 981.

This statement is of greater general importance than most people realize. It testifies to the fact that this new class of efficient drugs has not only been a financial boon to retail pharmacy but a very effective morale builder. The fact of his being the responsible dispenser of drugs of undeniable efficacy has fostered the professional feeling of the pharmaceutical practitioner and has served as a stop signal to the over-commercialization of the retail drug trade. In other words, the tendency toward changing the "drugstore" into a retail "pharmacy" has received an enormous uplift through the antibiotics; and from the initiative of a few progressive individuals has grown a real movement in this direction.

So far as pharmacy and—to some extent—medicine are concerned, there is no longer room for the depressing and demoralizing drug nihilism of the Viennese school of pathological anatomy that spread all over the world during the last part of the nineteenth century and lingered on up to the first chemotherapeutic deeds of Paul Ehrlich. The time is definitely over when the famous statement by Oliver Wendell Holmes could be made (1860): "If the whole materia medica, *as now used,* could be sunk to the bottom of the sea, it would be all the better for mankind—and all the worse for the fishes."[37] We still do not know what such an action, undertaken today, would mean to the fishes; but we certainly know what an immeasurable misfortune it would mean to mankind.

[37] Robinson, V. *The story of medicine.* New York, New Home Library, 1943, p. 476.

Notes and Queries

Edited by Ralph H. Major, M.D.*

The Electron Microscope

Few physicians are familiar with the electron microscope. The following note as to its *early history* and the *general principle* on which it operates may be of interest.

In the *Journal of Applied Physics* for August 1943 (vol. 14, pp. 434-436), Reinhold Rudenberg, Gordon McKay Professor of Electrical Engineering at Harvard University, writes as follows: "Stimulated by a case of virus disease in my family and being aware of the discovery of my friend, Hans Busch, that certain magnetic and electric fields in space are capable of concentrating cathode rays in a focus, I recognized late in 1930 the possibility of making use of electrons for producing greatly magnified images of small objects, with the purpose of advancing into the domain of viruses. Subsequently I realized that electrons can be caused to produce a real image of a physical object, forming a true pattern of its contrasts, and that such electronic image can be magnified further by repeated application of electro-magnetic fields, shaped to act like a lens. This was partly an analogy to the use of optical microscopes and similar magnifying instruments, but with due emphasis on the inherent properties of electrons as different from light waves." For this invention of the electron microscope Dr. Rudenberg received in 1946 the Stevens Honor Award Medallion. The following Figures 1 and 2 are taken from his original disclosure.

The principle on which the electron microscope operates is explained in Figure 1. The cathode ray gun is the source of electrons. The application of a set of magnetic or electrostatic lenses makes the cathode rays converge or diverge. In this way, analogous to optic lenses, magnification of the object is brought about and the enlarged image is projected upon a fluorescent screen. Further magnification may be caused by the use of an optical microscope (light-magnifier). Figure 2 shows different types of magnetic or electrostatic lenses as they are built into the electron microscope. A comparison with the optical microscope will serve for further elucidation (Fig. 3).

The power of resolution of microscopes is determined by the minuteness of the wave-length of the illuminator. In optical microscopes, light waves of the magnitude of 10^{-5} centimeters are used. Electrons of the electronic microscope have a diameter of less than 10^{-12} centimeters and, according to the quantum theory, their related wave length is still of the order of 10^{-8} centimeters. There-

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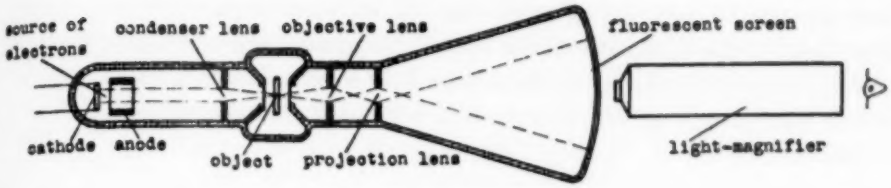
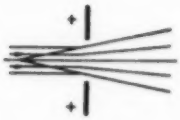


Electron Microscope

Fig. 1

Electron Lenses

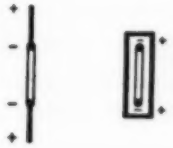
short coil, forming magnetic lens



positive, diverging lens

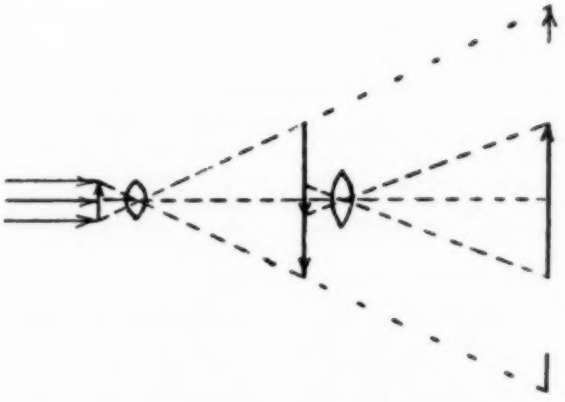
apertured plate, forming electrostatic lens

negative, converging lens



neutral electrostatic lenses

Fig. 2



compound microscope Janssen, 1590

Fig. 3

fore, contrary to the belief of many that the medium of glass for magnifying lenses caused the limitation of the optical instrument, the essential difference between an optical and electron microscope is the source of illumination: electrons with a wave length of 10^{-9} as contrasted with light waves of the order of 10^{-5} centimeters.

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Addenda on Petrus de Montagnana

The identification of the Petrus de Montagnana whose portrait graces the *Fascicolo di medicina* of 5 February 1493/94[1] remains uncertain. Query No. 5 of this department[2] sets forth some of the problems. Dr. Charles Singer in 1946[3] could see no solution. It is the purpose of this note to examine a related identification made many years earlier in a political journal and not easily accessible to historians of medicine.

In the years 1881 and 1882 the Neapolitan historian Camillo Minieri-Riccio was publishing a series of biographies of members of the Accademia Pontaniana. They included a short account of a Pietro Montagnana,[4] learned in Hebrew, Arabic, Greek, and Latin, who died in 1478 and bequeathed his rich library of manuscripts to the canons of S. Giovanni in Viridario in Padua. Minieri-Riccio quoted from Pietro Angelo Spera on the library and identified Montagnana with the author of the treatise on urines published in the *Fasciculus medicinae* of 1491[5] and of a letter to the physician Antonio Galateo which will be cited below.

Pietro Angelo Spera of Pomarico was a learned ecclesiastic who lived from 1597 to 1665.[6] He was the author of several works, two of which exist today in printed form, the *De passione D. nostri Iesu Christi Virgiliocentonis libri quattuor*[7] and the *De nobilitate professorum grammaticae et humanitatis utriusque linguae libri quinque*.[8] On pages 425–426 of the latter Spera draws on a work by Bernardino Scardeonio[9] which in turn gives a fuller account of Montagnana's learning and his library. His manuscripts, especially those in Hebrew, were such

[1] Venice, Joannes and Gregorius de Gregoriis.

[2] *J. Hist. Med.* 1946, *1*, 337–338.

[3] Singer, C., and Rabin, C. *A prelude to modern science*, Cambridge, University Press, Published for The Wellcome Historical Medical Museum, p. xxxi, §.

[4] I have worked from photostats of the two pages on Montagnana in the copy of the collected biographies in the Bibliothèque Nationale, *Biografie degli accademici alfonsini, detti poi pontaniani, dal 1442 al 1543* (s.l.n.d.), in-16, viii, 620 pp. The ascription of a copy to the British Museum in Query No. 5 seems unfounded.

[5] Venice, Joannes and Gregorius de Gregoriis.

[6] I am deeply indebted to Dottoressa Guerriera Guerrieri, Direttrice of the Biblioteca Nazionale in Naples, for information on Spera from Nicolò Toppi, *Biblioteca napoletana . . .* (Napoli, 1678) p. 250; Camillo Minieri-Riccio, *Memorie storiche degli scrittori nati nel regno di Napoli* (Napoli, 1844) p. 335; and Giuseppe Gattini, *Saggio di biblioteca basilicatese* (Matera, 1908) pp. 49–50, no. 428.

[7] Naples, 1647. The copy in the Harvard College Library has a presentation note by the author.

[8] Naples, 1641.

[9] *De antiquitate urbis Patavii et claris civibus patavinis libri tres in quindecim classes distincti* (Basel, 1560) fol. 243–244. For information from this work and from the one cited in Note 8 I am again indebted to Dottoressa Guerrieri.

a marvel to his biographers that they could hardly believe them the accumulation of a single individual. Enriched by his annotations and scholia they brought honor for many years after his death to the library of S. Giovanni in Viridario. Many of them, scattered today, can still be identified by the inscription "Eximius grammaticae doctor, Latinae Graecae et Hebraicae peritiss. Petrus Montagnana sacerdos, optima fide, pietatis studio, proque animae salute ascripsit, donavitque Canonicis Regularibus, qui Patavii morantur, hunc librum. Quicunque leget, proficiat: deinde sit gratus. Anno Domini M.CCCC.LXXVIII."

Jacopo Filippo Tomasini[10] provides a list of the manuscripts of S. Giovanni in Viridario which happily distinguishes those which belonged to Montagnana. They number some ninety, include exactly the items which one might expect a grammarian of Latin, Greek, and Hebrew to have, and reveal no special interest in medicine on the part of their owner. Unless he had medical manuscripts which went elsewhere, it seems necessary to conclude that he was priest and grammarian only, not physician, and not the author of the *De urinis*. Moreover, the letter to the physician Antonio Galateo,[11] also a member of the Accademia Pontaniana,[12] is occupied with fulsome praise of Roberto Caraccioli and contains no medical allusion whatsoever.

The fact remains that the Petrus de Montagnana pictured in the *Fascicolo di medicina* is surrounded by medical books of Arabic, Greek, and Latin authorship. The selection accords well with the knowledge of languages mentioned above. On the other hand, no other selection of truly representative medical authors was possible for the period, and the works originally written in Arabic and Greek could be obtained in Latin translation. We need stronger evidence to allow us to resolve into one individual the Montagnana of the *De urinis* and/or the portrait and the Montagnana of the library.

Cleveland, Ohio DOROTHY M. SCHULLIAN

Medical History in Foreign Publications

A. Pazzini: Il contributo dei Sanitari ai moti del Risorgimento (fino al '48-'49). [Contributions of medical men to the Risorgimento (to 1848–1849).] *Annali di Medicina Navale e Coloniale*, 1949, *54*, 137–153.

G. Pezzi: Alessandro di Tralles visto da un medico militare. [Alexander of Tralles as seen by a military doctor.] *Annali di Medicina Navale e Coloniale*, 1949, *54*, 154–163.

[10] *Bibliothecae patavinae manuscriptae publicae et privatae* (Udine, 1639) pp. 10–40. I have used microfilm from the copy in the University of Chicago Library. See also R. Sabbadini, *Le scoperte dei codici latini e greci ne' secoli XIV e XV* (Florence, 1905) pp. 62, 187, and Seymour de Ricci, *English collectors of books and manuscripts* (Cambridge, 1930) p. 42.

[11] Domenico de Angelis, *Le vite de' letterati salentini* (Florence, 1710–13) p. 24.

[12] See *Archivio storico per le provincie napoletane*, 1880, *5*, 364; and Galateo's *Liber de situ Iapygiae* (Basel, 1558) p. 4. A check of his letters in Angelo Mai, *Spicilegium romanum*, 1842, *8*, 523–608, discloses none to Montagnana.

G. Pezzi: Un precursore della storia della medicina navale Andrea Corsini. [Andrea Corsini, a forerunner in the history of naval medicine.] *Annali di Medicina Navale e Coloniale,* 1949, *54,* 164–170.

G. Pezzi: I grandi navigatori del Cinquecento e gli albori della medicina tropicale. [The great navigators of the sixteenth century and the beginnings of tropical medicine.] *Annali di Medicina Navale e Coloniale,* 1949, *54,* 513–518.

G. Rizzi: Medicinali e medici sulle navi della repubblica di Venezia. [Medicines and physicians in the Navy of the Venetian Republic.] *Annali di Medicina Navale e Coloniale,* 1950, *55,* 95–108.

Luigi Belloni: Pestilenza e contumacia nelle oselle di Venezia. [Plague and quarantine in the Venetian *oselle*]. *Annali di Medicina Navale e Coloniale,* 1950, *55,* 379–383.

Aroldo Baffoni: Prima descrizione della tisi bovina nel "De re rustica" di Columella. [The first description of bovine tuberculosis in the *De re rustica* of Columella.] *Archivio di Tisiologia,* 1950, *5,* 2–4.

Aroldo Baffoni: Antichi metodi di cura della tubercolosi. [Older methods of treating tuberculosis.] *Archivio di Tisiologia,* 1950, *5,* 167–175.

Aroldo Baffoni: Un grande studioso e vittima della tubercolosi: Giacinto Laënnec. [A great student and victim of tuberculosis: Hyacinthe Laënnec.] *Archivio di Tisiologia,* 1950, *5,* 83–86.

Aroldo Baffoni: Origini e vicende del concetto di contagio tubercolare attraverso i tempi. [Origins and vicissitudes of the concept of tuberculous contagion through the ages.] *Archivio di Tisiologia,* 1950, *5,* 216–222.

V. Lubich: Come era visto il problema del contagio tubercolare nella prima metà dell' ottecento. [The problem of tuberculous contagion as seen in the first half of the nineteenth century.] *Archivio di Tisiologia,* 1950, *5,* 255–257.

A New Society of Medical History in Canada

A recent issue of *Canadian Doctor* has announced the formation at Quebec of a society to be devoted to the history of medicine. Dr. Charles Vézina, dean of the Faculty of Medicine at Laval University, is honorary president, and Dr. Sylvio Leblond, professor of medical history at Laval, is president. "The society will first prepare a history of the medical faculty at Laval for publication in connection with the university's forthcoming centenary celebrations. It will then take up a study of the history of medicine in French Canada and eventually widen its work to cover the history of Canadian medicine."

Israel Society of Medical History

A communication dated at Jerusalem 27 May 1951 and addressed to the *Journal* will be of interest to our readers.

The Congress of the Society of Medical History in Israel dedicated its first session on 2 May 1951, at Tel-Aviv, to the memory of the celebrated English physician, Thomas Hodgkin, who accompanied Sir Moses Montefiore twice to Palestine and died there on his second journey in 1866. Dr. S. A. Plashkes, one of the executive members of the Society, delivered an address about the life, scientific work, and philanthropy of Hodgkin, recalling that together with Bright, Addison, etc. he was one of the founders of English pathological medicine, that he first described the lymphogranulomatosis known as Hodgkin's disease, and also described aortic insufficiency prior to Corrigan.

The Congress then went to Hodgkin's grave at Jaffa and paid tribute to the memory of that great clinician and noble friend of Jews and humanity alike, and placed a wreath of flowers on the tombstone erected by his friend, Moses Montefiore.

Brazilian Institute of the History of Medicine

A circular letter received from the Brazilian Institute of the History of Medicine announces that it has been legally recognized by the Brazilian Congress as an Association of public utility.

The first Brazilian Congress of the History of Medicine was held at Rio de Janeiro 14–21 July 1951.

Dr. Chauncey D. Leake Gives Clendening Lectures

The second series of lectures under the Clendening Lectureship on the History and Philosophy of Medicine has been given by Dr. Chauncey D. Leake, Vice President of the University of Texas—Medical Branch. On 24 April he spoke on "New Generalities From Old Egyptian Medical Papyri" and the following day on "The Hearst Medical Papyrus."

The Lectureship was established in 1949 at the School of Medicine of the University of Kansas by Mrs. Logan Clendening in memory of her husband, and Dr. John F. Fulton gave the initial series of the lectures in November, 1949.

Dr. George Rosen's Appointment at Columbia University

Dr. George Rosen has been appointed Professor and Head of the Department of Public Health Education at the School of Public Health of Columbia University. The appointment is effective as of 1 September 1950.

Appointments at the Institute of the History of Medicine

Richard H. Shryock, Director of the Institute of the History of Medicine, The Johns Hopkins University, has just announced the award of three fellowships in the History of Science and of Medicine for the academic year 1951–1952. Those receiving the fellowships for next year are Mr. John B. Blake, of Harvard (in the history of public health); Mr. R. Gordon Gilbert, of the University of California (in the history of basic scientific concepts); and Mr. Rashi Fein, of Johns Hopkins University (in medical economics).

Announcement has also been made of the appointment of Professor Alexandre Koyré, of L'Ecole Pratique des Hautes Etudes in Paris, as Visiting Professor in the History of Science for the first semester, 1951–1952.

Book Reviews

DOROTHY M. SCHULLIAN AND FRANCIS E. SOMMER. *A Catalogue of Incunabula and Manuscripts in the Army Medical Library.* New York: Published for the Honorary Consultants to the Army Medical Library by Henry Schuman, Inc. [1950]. xiii + 361 pp., illus. $15.00.

THE publication of a rare-book catalogue by one of our leading libraries is always an event in the bibliographical world. In the present instance, the publication of *A Catalogue of Incunabula and Manuscripts in the Army Medical Library* is also an event of importance in the medical world, for the texts of the early printed books and manuscripts in its collections range from the writings of Hippocrates, the Father of Medicine, to Michele Savonarola, who wrote on thermal baths and fever in the fifteenth century—a scope of some nineteen hundred years.

Of the 493 incunabula in the collection, at least 167 are first editions. Four hundred twenty-two are included in Dr. Klebs' *Incunabula Scientifica et Medica.* More than 50 are the only registered copies in North America, and many others are so rare, they are known in only two or three copies. As for the manuscripts, each is unique in its own right. The Arabic collection is exceptional in its size and rarity. One of its most precious manuscripts is a sixteenth-century rendering of the *Canon of Medicine* by Avicenna (980?–1037), containing the seldom-found sequence of all five books of his famous work. Of the twelve illustrations in the *Catalogue,* five are from the Library's manuscript collections. Of the seven taken from its incunabula, five are the only registered copies in the country, and one is not in Klebs.

It is obvious that the Army Medical Library's collections, which have been brought together during the last eighty years through the acumen of its various Librarians, are comprehensive and rich in manuscripts and first-printed books, whose importance to medical history is unquestioned. The books, as the result of the long-term restoration program described by Dr. Max H. Fisch in the Preface, are in prime condition, and microfilms of all of them have been made. The *Catalogue* itself, which has been skillfully compiled and organized by Dr. Dorothy M. Schullian and Francis E. Sommer, is a work of high order. It has been published for the Honorary Consultants to the Army Medical Library by Henry Schuman, Inc. of New York.

The *Catalogue* is divided into two parts. The first, compiled by Miss Schullian, includes both incunabula and such of the Library's collection of Western Manuscripts as were produced prior to the incunabula period, or correspondent to it in the fifteenth century—a total of 529 entries in single notation (four of which were added after the notation had been fixed). This section also includes an historical Introduction of great interest; a list of Abbreviations; Concordances

to Klebs' *Incunabula Scientifica et Medica* and Stillwell's *Incunabula in American Libraries;* and a General Index listing printers, places, owners, variant forms of authors' names, and a chronology of imprints.

The second part of the *Catalogue,* the work of Mr. Sommer, relates entirely to Oriental Manuscripts. Following his Introduction, the notation divides into five series, each section with an identifying initial accompanying the notation, in order that one may distinguish at a glance whether a given manuscript is written in Arabic, Persian, Turkish, Singhalese, or Hebrew. There are 137 entries, of which 92 are Arabic. One cannot but admire the linguistic ability of the compiler, who seemingly handles these texts with ease. So far as possible, he has trained each entry to follow the same pattern. The title in the vernacular is followed by its transliteration in Western characters, and often by its translation in English. Notes describe the general subject-matter of the manuscript, the author and his dates, the condition of the manuscript and its dates known or assigned; references are given and whatever factors are peculiar to the manuscript itself. Even if one cannot read Singhalese or Arabic—and not many of us can—it is a delight to browse among these strange titles and to discover from their notes that one is a collection of 32 treatises on alchemy by an eighth-century author; another, a sixteenth-century treatise on eye disease; still another, a treatise on hygiene for travelers by a later physician. One feels something of the personal equation in the manuscripts through these brief annotations. The section ends with an Index of transliterated titles and an Index of names. The book as a whole closes with the list of pre-publication subscribers.

It is easy to see that much solid work has been done behind the scenes in all sections of the book. The study of incunabula, for instance, is so intricate and involved that it is only through such sound scholarship as Miss Schullian possesses that bibliographical notes can be compressed to a minimum. And it is merely by a simple statement in her Introduction—that she has recorded only "variations from descriptions already existing"—that she modestly reveals the infinite labor of collating each volume with its bibliographical description in accredited reference works, to see whether or not variations exist. The result of skillful elimination is that the entries are concise and to the point. In addition to such occasional variations as are noted, each entry includes author, brief title, and imprint. Added titles included in a given book are noted. Reference numbers to Klebs and Stillwell are given and, whenever possible, to the British Museum's *Catalogue of Books Printed in the XVth Century,* to the *Gesamtkatalog der Wiegendrucke,* and other bibliographies or monographs. The size of the copy is given; its binding, marginalia, and annotations are described. In other words, all of the data essential to the proper identification of each book are given and no more. Clarity is enhanced by the intelligent type selection of the publisher and of the Anthoensen Press, long experienced in the gentle art of making typography the handmaiden of bibliography.

There is a sense of sanity in the *Catalogue*. It sets out to do certain definite things, and it does them. In the incunabula section, it goes so far and no farther. The names of translators, editors, and commentators are omitted. There is no attempt at classification nor subject analysis. Such matters are left for a later time.

The need for subject analyses and interpretations is ably stated in Dr. Fisch's Preface. "The great desideratum," so he writes, "is analyses and interpretations of the contents of the volumes. Incunables in particular have long been studied from the point of view of format, typography and presswork, paper, watermarks, printers' marks, colophons, bindings and other relatively external features. What we need to know for purposes of medical history is what is in the texts." And later he adds, "The books printed before 1501—the incunables or cradle books—are a group of particular interest. They include not merely the current literature of the last third of the fifteenth century, but also the first printed editions of the medical classics of the nineteen centuries from Hippocrates onward, which had previously circulated in manuscript form. . . . Clinical and experimental research constantly discloses new truth, which gradually finds its way into the textbooks. But medicine is an old art and science. Not all medical truth is new or recent, and not all old truth is kept alive; it is always dropping out of the textbooks to make way for new. Historical research restores to life old but forgotten knowledge and sometimes makes it as good as new."

The task of finding what is in the texts of the first-printed medical books will of necessity be a long one. It belongs equally to the bibliographers and the members of the medical profession. Miss Schullian, in the present *Catalogue,* has taken care of the technical, bibliographical aspects of these early books, once and for all. The field is thus clear for action.

In her Introduction she tells of the work originally done on the collection by Dr. Arnold C. Klebs, and of the three short-title lists of the Library's incunabula published respectively by Col. McCulloch, Dr. Garrison, and Dr. Mayer in the years before the present definitive volume was undertaken. It may well be, therefore, that the second part of the project—the classification and analysis of the incunabula texts—can similarly be best accomplished in successive stages. A short-title list (with the same notation and references as the present volume, but with all bibliographical notes omitted) might, for instance, give brief notes on the author, translator, editor, and commentator, together with a brief statement of the subject-content of each incunabulum, such as Mr. Sommer gives for the Oriental Manuscripts. Thus the texts would be placed historically in their respective centuries and, if listed in a classified index, they would be grouped and ready for further and more intensive study and interpretation.

But however it may be done, one may rest assured that the task eventually will be done, and done well. For a Library that has had the tenacity to keep to the project suggested in part by Sir William Osler in 1913, having now achieved so

much of its objective, will not rest content at that, eminent though its present achievement may be. We can only hope that this matter of subject analysis and interpretation, discussed in both Dr. Fisch's Preface and Miss Schullian's Introduction, may soon be under way. Meantime, the book in hand is in itself a definite contribution to the history of medicine.

Providence, Rhode Island MARGARET BINGHAM STILLWELL

GEORGE URDANG. *The Scope of Pharmacy*. Madison, Wisconsin, American Institute of the History of Pharmacy, 1946. 61 pp., illus.

DESCRIBING an exhibit arranged in 1941, when the American Institute of the History of Pharmacy was established at the University of Wisconsin, this account was first published in the October 1945 issue of the *American Journal of Pharmaceutical Education*.

The description of the exhibit is offered as a pattern and as an encouragement. The brochure includes Doctor Urdang's address and an account of books and other material displayed relating to European pharmacy, American pharmacy, and general aspects of pharmacy that were on display. There is also described significant items relating to important leaders in pharmacy.

The exhibit was most carefully arranged and contained a surprisingly rich collection of material from Graeco-Roman to modern times. The brief bibliographical descriptions of the works shown make the book particularly valuable.

Galveston, Texas CHAUNCEY D. LEAKE

The Proceedings of the Charaka Club. Volume XI, New York, Richard R. Smith, 1947. ix + 243 pp. $7.00.

THE CHARAKA CLUB was founded in 1898 by Charles L. Dana, Joseph Collins, Frederick Peterson, and Bernard Sachs. According to Dana they were interested in the literary, artistic, and historical aspects of medicine, but soon felt that subjects beyond the range of medicine would prove attractive.

Over the years there have been about seventy members. The present membership is about twenty-five. The list contains many distinguished names in American medicine.

The present volume consists of twenty-four pieces, one third of which deal with past members of the club. Archibald Malloch's reminiscences of Samuel W. Lambert, Rufus Cole's article on Walter R. Steiner, and Fred B. Lund's article on Casey A. Wood are all excellent and lead the reader to wish they were longer.

The remaining pieces cover a wide range and include translations into verse of some of the Odes of Horace by Fred B. Lund, an essay entitled "What is Time," by Karl Vogel, "The Lamentable Decline in Self-Satisfaction" by Peyton Rous, and "On Burning Old Letters" by Rufus Cole.

An article entitled "The Past, Present and Future of the Charaka Club" by Joseph Collins makes it very clear that the Club is not a medical society and especially not a medical history society. According to Collins "more and more so-called medical history crept in." Your reviewer having read some of the earliest volumes would suggest that more and more medical history has crept out. The early volumes contain many excellent historical papers. The present volume, except for the biographical material, contains nothing of medical historical interest. A club which numbered among its past members Billings, Garrison, Osler, Mumford, and Pilcher must have had at one time a deep interest in medical history.

However, any club which encourages physicians to cultivate wide cultural interests and avocations is performing a very worthy function indeed and its pieces should not be reviewed with imperfect sympathy.

New York City CLAUDE E. HEATON

GEORGE SARTON. *The Life of Science*. New York, Henry Schuman, 1948. vii + 197 pp. $3.00.

IT is a pity that American publishers are generally unwilling to publish collections of essays which originally appeared in a scatter of periodicals. The British are more enterprising, and to their initiative the *conoscenti* who prize the essay as the best instance of *multum in parvo* are much beholden. Now in the ambit of gratitude can be included the American publisher, Henry Schuman, who has issued over his imprint a volume of eleven of George Sarton's essays, entitled *The Life of Science*.

The substance of this volume has been drawn from thirty years of devoted and enthusiastic exposition of the history, scope, purpose, and method of science. It iterates Sarton's prime article of faith—"the rhythm of mutual interrelations between science, art and religion." "Science is the reason, art the joy, religion the harmony of life. None is complete without the others. We cannot hope to understand the mystery of life unless we be prepared to consider it from these three angles and this means first of all that we must drop our scientific conceit, and second, that we must never, never subordinate humanities to technicalities." (p. 28)

Sarton is a highly motivated person, in the very same sense that Socrates was. And like him, Sarton has an inordinate faith in the power of knowledge and reason over the behavior of men and nations. He does describe the history of science as amounting in the main to "psycho-sociological investigation," but in effect the *psycho*-portion of this triad finds little representation in the argument of his essays. But one should read these essays to relish them rather than to dispute them. And indeed there is little any reasonable and tolerant person will want to dispute, and a great good deal that he will want to underscore for rereading and citation. Sarton can and does turn many a neat phrase embodying the essence

of wise insight and experience. "Nothing excites the mind more than a return to the sources." "It is the historian's main duty to revive the personalities, rather than to enumerate their scientific excressences." "One of the main functions of the history of science is . . . to give us, who are the moderns of today, a less conceited view of our share in the total of human evolution."

The essays are divided into four groups; the first is devoted to the spread of understanding, then follow four biographical essays, preceded by a brief excursion entitled 'Secret History.' The burden of this prelude is that "The history of mankind is double; political history which is to a large extent a history of the masses, and intellectual history which is largely the history of a few individuals." Even with the conditioning terms "to a large extent," and "largely" this is a weak and dubious proposition, and is one of the least persuasive of those advanced by Sarton. Of the four biographical sketches included in this section, those on Leonardo and Evariste Galois are the most appealing and sympathetic. The other two are of Ernest Renan and Herbert Spencer. Sarton makes a brave attempt to render Spencer a sympathetic personage, but does not succeed too well, and himself resigns the effort, making the occasion a plea for "synthetic science." In "East and West in the History of Science," Sarton beautifully illuminates the debt of modern, Western science, to its great and all-too-little appreciated Eastern precursors. In the fourth portion Sarton pleads eloquently, almost passionately, for an Institute for the History of Science and Civilization. Such an Institute, he argues, would by the humanization of science serve as an insurance against its gradual barbarization. He might have added with equal warrant that such an Institute by the humanization of *the history of science* would likewise save it from the menace of a similar corruption. Not a few among the modern "historians" of science are rewriting its records in terms of pure "economic and rational" determinism. The result is the dehumanization of the history of science.

All of Sarton's essays are stimulating; one in addition is provoking. It calls for a protest. This is the second essay in the list entitled "The History of Medicine versus the History of Art." There is good reading in this essay, but it embraces and elaborates a fallacious thesis. Sarton maintains that "Science is progressive and therefore ephemeral; art is nonprogressive and eternal." In support of this thesis Sarton quotes Picasso, who is without doubt a great painter, but hardly an historian of art, and no such philosopher of art as to command concurrence for his judgment. I'd rather be instructed by Roger Fry. In his Last Lectures, Fry sets science and art in apposition as follows. "Science concerns the relations of man to his environment. Art is purely a family matter among human beings." But art is, according to Fry, as progressive as any of the sciences. How else could it have a history? That which is static can at best have only a catalogue.

But this is all too large a subject to expound at the tail end of a review, the primary objective of which is to report how much pleasure, instruction, and satis-

faction is to be gained from this volume of collected essays, written by a great historian, a great scholar, and an inspiringly warm humanist.

New York City IAGO GALDSTON

WILLIAM H. WOGLOM. *Discoverers for Medicine.* New Haven, Conn., Yale University Press, 1949. x + 229 pp., illus. $3.75.

A QUOTATION from Oliver Wendell Holmes by the author describes the subject matter of this volume:

> "Medicine . . . learned . . . from a Jesuit how to cure ague, from a friar how to cut for stone, from a soldier how to treat gout, from a sailor how to keep off scurvy, from a postmaster how to sound the Eustachian tube, from a dairy maid how to prevent smallpox, and from an old market woman how to catch the itch insect."

The thesis is that some "amateurs" have made scientific discoveries that have led to notable advances in medicine. Selected chapters are used by the author to illustrate his point—Rev. Stephen Hales and blood pressure; Antoine Laurent Lavoisier and respiration; William Withering and the foxglove; Edward Jenner and vaccination; Manuel Garcia and the laryngeal mirror; Edmé Gilles Guyot and the Eustachian tube; Benjamin Franklin and eyeglasses and spectacles; Simon François Renucci and the itch; Elie Metchnikoff and phagocytosis; Wilhelm Conrad Roentgen and X-rays; Gregor Johann Mendel and heredity; and John Rowe and milk sickness.

As will be apparent, the author has been eclectic in the treatment of the subject. Each phase selected, however, is clearly and interestingly presented. He contends "that medicine has often done no more than put the seal of its approval on folk beliefs" but "medicine has not taken all and returned nothing." He cites the contributions made by medicine to etching, music, literature, politics, and science.

There is a good bibliography supporting each of the chapters, and the index is complete. Fourteen illustrations serve to enhance the text. To anyone interested in the history of medicine this volume throws light on many of the discoveries in medicine that have had enduring benefits to man.

Columbus, Ohio ROBERT G. PATERSON

Autobiography of Dr. Robert Meyer. With a Memoir of Dr. Meyer by Emil Novak, M.D. New York, Henry Schuman, Inc., 1949. ix + 126 pp. Frontispiece portrait. $2.50.

THIS autobiographical sketch first appeared in serial form in the *Journal of the History of Medicine and Allied Sciences* under the title "A Short Abstract of a

Long Life." In the present volume, in addition to the original text, there has been added a memoir by Dr. Emil Novak, a bibliography of the works of Dr. Meyer, a table of his classification of ovarian tumors, and an index.

This book has a two-fold interest, in its appeal to physicians in general as well as to the specialist. It contains the recollections of a truly great man, a physician of broad culture, the first seventy years of whose life comprised a happy childhood and many serene decades of hard work as a scientist and teacher. These were the years when German medicine was at its best. Finally, in his sunset years Dr. Meyer was driven into exile by the Nazis and at the age of seventy-five came to the United States where he spent the last eight years of his life. One regrets that Dr. Meyer did not give us more about his interesting life rather than details of his work in general pathology.

However, those of us who are interested in gynecology and obstetrics will find much of interest because of Dr. Meyer's contributions to embryology and pathology. Beside his great contributions to the field of embryology he did outstanding work on ovarian tumors, especially certain special tumors and their relation to sex characteristics. He was also a pioneer in the field of cancer diagnosis, showing that it was possible to find carcinoma of the cervix in its pre-invasive phase.

The bibliography lists about two hundred contributions to the literature.

New York City CLAUDE E. HEATON

MAX H. FISCH. *Nicolaus Pol Doctor 1494.* With a Critical Text of his Guaiac Tract Edited with a Translation by DOROTHY M. SCHULLIAN. New York, Herbert Reichner, 1947. 244 [2] pp. $12.50.

To any scholar, be he of science or of the humanities, the library of a discriminating collector brought together at the dawn of printing and in the heyday of the Renaissance is a subject of challenging interest. In the present study such a library is described by Dr. Max H. Fisch, himself an eminent scholar, and he has spared no pains to locate and describe all known items in the original collection of "Nicolaus Pol Doctor." This work was undertaken by Dr. Fisch, now Professor of Philosophy at the University of Illinois, when he was Director of the Rare Book Division of the Army Medical Library in Cleveland. The volume was planned to celebrate the semi-centennial in 1944 of the Cleveland Medical Library Association which possesses a notable portion of the Pol library, but was delayed because the invasion of Italy prevented communication with those Italian libraries where it was known that other of the Pol books were to be found.

In 1929 Maggs Brothers, the well-known book dealers of London, issued a catalogue of something less than forty incunabula and early sixteenth century books bearing the characteristic inscription "Nicolaus Pol Doctor 1494"; the

catalogue stated that there might one day be sufficient material for a monograph on "the distinguished collector of these books, but at present pitifully little is known." Since that time, scholars, collectors, librarians, and bookmen have searched diligently for material about Pol, and their joint efforts, which have been here gathered by Dr. Fisch, have yielded significant information about the man and his library.

Of Pol's parentage or the date and place of his birth nothing is known. He was probably born in the Tyrol about 1470. It is known that in 1487 he entered the service of the Duke Sigismund at Innsbruck as court distiller and pharmacist with the paltry stipend of twenty florins a year. When or where he studied medicine is not clear, but among the physicians at court during Pol's service were Burkhard von Horneck, Conrad Heingarter, Ulrich Ellenbog, and Adolf Occo, all four of whom may have contributed in some measure to Pol's study of medicine. And it would be interesting also to speculate on what influence Ellenbog's magnificent library had on the aspiring young medical student.

In 1495 Pol was advanced to the position of personal physician to Maximilian and his governors at Innsbruck. As physician, Pol's salary was increased to one hundred florins a year, but one must assume from the character of his library that he either had private means or that he augmented his official stipend through private practice.

Of Pol's library some 467 items have been definitely traced, many of which were published prior to 1500. These are listed in Dr. Fisch's compilation. It is probable, however, that the library contained many other items. In 1907 the late Dr. Edward Clark Streeter of Boston purchased thirty volumes from the Pol library; these had been discovered by a new employee of Ludwig Rosenthal's Antiquariat, Maurice Ettinghausen by name, as he was going through the stock in the process of learning the business. Twenty years later Mr. Ettinghausen, then of Maggs Brothers, bought back these books from Dr. Streeter for $5,000 and, after adding three other incunabula, issued a catalogue offering the lot for £2,500. Through an oversight Harvey Cushing did not receive this catalogue, but his nephew Dr. Edward H. Cushing of Cleveland did, and he promptly cabled for the collection, thus beating his uncle to the draw. These 33 items, to which five more were later added through Edward Cushing's efforts, comprise the collection in the Cleveland Medical Library Association and have been described in detail by Dr. Fisch. Also included are thirteen additional items from Pol's library subsequently obtained by Dr. Harvey Cushing which are now in the Historical Library at Yale. Eight of these are incunabula.

By far the largest part of Pol's library resides in the Collegiate Church of San Candido (near Innichen). They possess 133 volumes containing 169 out of a total of 272 incunabula and 166 out of 309 books of the first two decades of the sixteenth century; also 25 manuscript volumes. Fortunately, these books came safely through the war.

Included in Dr. Fisch's volume, in addition to his notes on Pol and his library, his sections on the Cleveland Collection and the Yale Collection, and his inclusive list of the books and manuscripts known to have belonged to Nicolaus Pol, is the Latin text, together with an excellent English translation by Dr. Dorothy M. Schullian, of Pol's tract on the guaiac treatment of syphilis dated 19 December 1517 but first printed in 1535 in Venice. This translation is based primarily on five folios of a manuscript in the Army Medical Library. Dr. Schullian chose to work from the manuscript rather than from the printed text of 1535 "because it is earlier . . . often fuller, and would seem therefore to be more faithful to the archetype."

Nicolaus Pol Doctor 1494 has been beautifully printed in the best bibliographical traditions by the Anthoensen Press.

New Haven, Connecticut JOHN F. FULTON

Regimen Sanitatis Salernitanum. A cura di G. Barbensi. Florence, Leo S. Olschki, 1947. x + 27 pp. Lire 1000.

ONE of the most popular of all medical books is the famous *Regimen* of Salerno. It first saw the light of day in medieval times, and since then it has appeared in hundreds of editions, both manuscript and printed. Originally a didactic poem of several hundred verses, it eventually grew by accretion to several thousand verses. In addition, it has been the subject of many commentaries, the first of these written by Arnald of Villanova in the thirteenth century. Indeed, a history of personal hygiene could be written around the *Regimen sanitatis*.

This most interesting book is now reprinted with a brief introduction by Barbensi. The present volume is a reprint of a fifteenth century edition, of which a copy is preserved in the Biblioteca Nazionale Centrale in Florence. According to Barbensi, it is not mentioned in the ordinary bibliographical lists. The printing is good but the bookmaking is poor. Nevertheless, it is pleasant to see this hardy medical classic in still another edition.

GEORGE ROSEN

HERBERT BUTTERFIELD. *The Origins of Modern Science*. London, G. Bell & Sons, Ltd., 1949. x + 217 pp. $2.50.

IT is an unusual occurrence when a historian can move easily and competently across the various fields that have been the object of Herbert Butterfield's sensitive and scholarly attention. Professor of Modern History at Cambridge, Butterfield has in past books sampled judicially the statecraft of Machiavelli, dug deeply into the peace tactics of Napoleon, and described closely a segment of the reign of George III. He also has given attention to the historical novel, to the Whig interpretation of history, and most recently to Christianity. With *The*

Origins of Modern Science he clearly solidifies his position as one of the foremost of contemporary historical essayists.

In this volume Butterfield reproduces lectures delivered in Cambridge at the request of the History of Science Committee of Cambridge, of which committee he is a member. The Committee wisely chose a "general historian" to inaugurate its program. For Butterfield has brought to the problem of the rise of modern science a historical breadth that is evidently lacking in the single-volume surveys of the history of science that desperately try to detail in catalogue fashion the names and niches significant to the growth of modern science.

Butterfield's objective is two-fold: to evaluate the factors which brought about the scientific revolution and to estimate the importance of the revolution as a historical event in the history of Western culture. His thesis is epitomized as he describes the

> "so-called 'scientific revolution,' popularly associated with the sixteenth and seventeenth centuries, but reaching back in an unmistakenly continuous line to a period much earlier still. Since that revolution overturned the authority in science not only of the middle ages but of the ancient world—since it ended not only in the eclipse of scholastic philosophy but in the destruction of Aristotelean physics—it outshines everything since the rise of Christianity and reduces the Renaissance and Reformation to the rank of mere episodes, mere internal displacements, within the system of medieval Christendom. Since it changed the character of man's habitual operations even in the conduct of the non-material sciences, while transforming the whole diagram of the physical universe and the very texture of human life itself, it looms so large as the real origin of the modern world and of the modern mentality that our customary periodisation of European history has become an anachronism and encumbrance."

In the twelve lecture-essays that follow Butterfield brings under close observation the stages and events in the scientific revolution. Particularly gratifying to the historian of medieval science is Butterfield's choice of the decay of Aristotelean mechanics as the point of departure. This decay took place, at least in part, under the influence of the Parisian impetus mechanics of the fourteenth century. This Parisian mechanics is a subject that has received little attention in English outside of monographs in spite of the fact that the broad achievements of the schoolmen of the fourteenth century were described a generation ago by the French physicist and historian of science, Pierre Duhem. It is largely upon the work of Duhem that Butterfield has based his first lecture. It is unfortunate, but understandable, that Butterfield has not seen some of the more recent and fundamental studies in medieval mechanics (such as those of Anneliese Maier) which serve to correct, supplement, and reinterpret the work of Duhem.

The most serious omission of the first chapter is any reference to the activity in mechanics, particularly in kinematics, that took place at Merton College, Oxford, during the first half of the fourteenth century. These men of Merton invented a new vocabulary for the study of accelerated motion. They fashioned for the first time a concept of instantaneous velocity and devised the kinematic

theorem which describes a uniformly accelerated movement by its mean velocity. Furthermore, they appreciated the basic distinction between kinematics and dynamics. It is true that Butterfield mentions the kinematic theorem of uniformly accelerated motion, but following Duhem he assigns its invention to the French. Manuscript and other evidence conclusively shows its prior development at Merton.

Butterfield also seems to be unaware of the quasi-mathematical character of some of the mechanical discussions of the thirteenth and fourteenth centuries, for he repeats the oft-heard conclusion that a mathematical approach to physical problems awaited the general publication of the works of Archimedes in 1544. Now it is no doubt true that the study of Archimedes' works became wider spread after 1544 and that they served as the most important mathematical catalyst to the growing mechanics, but it should be noted that a closer examination of the physical treatises of the thirteenth and fourteenth centuries reveals that a revival of Hellenistic geometry including some of the works of Archimedes played a significant rôle in medieval mechanics.

Thus the use of Hellenistic geometry and its offshoot, Hellenistic mechanics, is particularly evident in a series of treatises on statics that circulated during the thirteenth and fourteenth centuries. The best of these treatises was an extended work entitled in some manuscripts *De ratione ponderis* and attributed to the mathematician Jordanus de Nemore. The form of the treatise is entirely that of Greek geometry, and its content, in some respects quite original, is also closely related to its Hellenistic predecessors. One of its most original propositions states with proof a correct conclusion on the equilibrium of weights on oppositely inclined planes. This conclusion is equivalent to the later sine law for determining the effective weight on an inclined plane.

Another interesting and important mechanical treatise of the thirteenth century which employed mathematical techniques was the *De motu* of Gerard of Brussels. Appearing in some of the same codices as the works of Jordanus, it seems to have been the first independent treatise on kinematics in the West. It assumes some importance when we realize that Thomas Bradwardine, who initiated the mechanical activity at Merton in the 1320's, had use of this treatise and quoted from it. The kinematics described by Gerard developed from what I have called generative geometry, problems that deal with the generation of areas by the movement of lines or volumes by the movement of areas. The form of Gerard's treatise is completely Archimedean, as is his constant use of the *reductio ad absurdum* or the *demonstratio per impossibile*. At the same time he cites with clear understanding Archimedes' *Quadrature of the Circle*, the Archimedean *De pyramidibus* (or *De curvis superficiebus*), and Euclid's *Elements*.

The importance of the revived mathematical tradition to the fourteenth century is clearly evident in (i) the fact that most of the manuscripts of the thirteenth century mechanical treatises stem from the fourteenth century, and

in (ii) the fairly generous quotation of the thirteenth century works by the authors of the somewhat more philosophical mechanical treatises of the fourteenth century. The point of this whole digression is simply to show that despite the general (and erroneous) impression of the completely non-mathematical character of medieval physics, there was, in fact, a distinctly mathematical element at the very points where medieval mechanics achieved some success.

After evaluating the importance of scholastic mechanics, Butterfield proceeds to a stimulating second chapter on "The Conservatism of Copernicus." The point of interest brought out by Butterfield is that Copernicus' system carried with it a dynamics that was neither medieval nor modern. Hence, the Copernican system did not yet represent the high point of the scientific revolution. For its success, the system had to await the development of a new mechanics. The only thing of significance I found wanting in the discussion on Copernicus was the medieval stage setting, the developments at Paris, Oxford, and Germany that made possible his facility with the tools of astronomy such as trigonometry.

From Copernicus, Butterfield turns to Harvey (Chap. III). He sets his discussion of Harvey against the rising tide of experimentation at the University of Padua. He rightfully points out that in medicine there was already by Harvey's time a real tradition of medical experimentation. Furthermore, it was precisely at Padua that we find the most advanced discussion of scientific method in the late middle ages. The "scholastic" medical men there were increasingly aware of the importance of observation and experiment in the obtaining of scientific knowledge. Butterfield lays particular stress on the quantitative considerations of Harvey. "An extraordinarily modern flavour attaches to his work as a result of the clearly mechanical nature of much of his enquiry and his argument; the importance that he gave to purely quantitative considerations; and the final cogency that he attributed to a piece of arithmetic."

But it is not so much in medicine that the scientific revolution was to be particularly evident, but rather in mechanics, in mechanics applied indifferently to celestial or terrestrial bodies. The resolution was not to come until Aristotelean physics as well as Ptolemaic astronomy had been swept away, and a new mechanics had been married to the new astronomy.

In discussing Galileo's contributions to mechanics Butterfield adopts Koyré's point of view. He assures us that we can not explain Galileo's success by the convenient answer that Galileo experimented. For as important as experimentation was for Galileo, it was his "geometrizing" of the problems of mechanics that was of even greater significance. We have already suggested that we first owe to the Hellenistic mechanicians the method of mathematical abstraction in physical problems, or if you will, the selection and manipulation of idealized quantities. The thirteenth century investigators recognized the power of this method and they used it with some success. But Galileo used it with greater skill than any of his predecessors, and yet, as Butterfield points out, his employment

of the geometrizing technique fell somewhat short of his successors (e.g., Newton). Butterfield repeats the finding of Koyré that Galileo's concept of inertia was not yet that of Descartes and Newton, his ideal inertial movement being circular rather than rectilinear.

It is, then, the skillful use of, and indeed the recognition of the paramount importance of, mathematical abstraction that sets a Galileo off from a Bacon, a successful mechanician from an empiricist. It is, of course, evident that abstraction must start from experience and must ultimately be able to return to experience for successful physical investigation. The most successful modern physical theories start from idealized postulates and erect upon them a systematic explanation by deduction, and such theories relate the deductive conclusions to experience by as many independent routes as possible. It was to the great glory of the mechanicians of the seventeenth century that they recognized the overwhelming importance of the mathematical-deductive procedure as well as the relationships that scientific theory must have with experience in the form of active experimentation. It is true, as Butterfield suggests, that Descartes laid too great a stress on the mathematical-deductive element without sufficient recourse to sophisticated experience, while Bacon, much less the mathematician than Descartes, emphasized the power of experimentation without a real understanding of the importance of the mathematical element (see Chapter VI, Bacon and Descartes).

Having to this point suggested the important methodological developments as well as some of the background factors in astronomy and mechanics, Butterfield then proceeds to an exposition of the climactic event in the scientific revolution, the development of the modern theory of gravitation. The key concepts of Newton's mechanics were (i) the inertial concept and (ii) the concept of a mutually attractive force of bodies. Butterfield has already said something about the development of the inertial idea through Galileo and Descartes. Now in Chapter VIII he undertakes a brief exposition of the history of gravitational ideas, mentioning the theories of Aristotle, Copernicus, Gilbert, Kepler, Galileo, Huygens, Hooke, and Newton. The various stages of the development of Newton's ideas are presented in rather traditional but satisfactory manner. It is, of course, difficult to present the relationships between Galileo, Kepler, Huygens, and Newton without recourse to the mathematical formulation involved, and any rhetorical account like that of Butterfield will necessarily suffer; but in view of the broader objective which Butterfield has taken, he can hardly be criticized for the thinness of his treatment of the mathematical aspects of the theory.

In some ways the last chapters of the book are the most interesting, for in them Butterfield attempts to trace the repercussion of the new science on various areas of thought in the seventeenth, eighteenth, and early nineteenth century. Thus in Chapter IX he finds in the writing of Fontenelle the link between the science of the seventeenth century and the *philosophes* of the Enlightenment.

Fontenelle emerges as the inventor of the technique of popularization as well as the creator of the legendary picture of the scientist that was adopted in the eighteenth century, the scientist who turned away from theology to geometry, from words to actions, to overcome the stupid conservatism of his age. "Fontenelle's biographical sketches, furthermore, give evidence concerning the curious social success of the sciences in the reign of Louis XIV."

As the course of popularization and philosophical interpretation continued into the eighteenth century, the *philosophes,* as Butterfield shows nicely, transformed the discoveries of the seventeenth century "into a new outlook and a new world-view." This new world view was crucial to the further development of Western civilization. Among other things the new view endorsed the tendency evident in Fontenelle to believe that science supplies the key to methodology in other areas of human endeavor like politics and economics. Thus the value of statistics for social life had already been demonstrated by Vauban and Petty, and Francis Bacon's faith in science as an aid to all branches of human development is often re-echoed by the encyclopedists and *philosophes* of the eighteenth century.

The synthesis of a wide variety of historical events with the scientific revolution presented in the tenth chapter will certainly interest the general historian and the historian of science alike. By his pen the scientific activity of the seventeenth century emerges as an essentially new factor in society despite the obvious continuity of its development, a factor that was to assume dominancy among the other permanent and important factors of our civilization.

The book is concluded in what strikes me as rather anticlimactic fashion by a not particularly original account of the delayed scientific revolution in chemistry in the eighteenth century (Chap. XI) and by a discussion of the ideas of progress and evolution and their relationship with the spread of modern science (Chap. XII). Both chapters seem rather like hasty afterthoughts. But they do not detract seriously from a book that will, I am sure, prove its value both to the general student and the specialist in the history of science.

Madison, Wisconsin MARSHALL CLAGETT

R. WALZER. *Galen on Jews and Christians.* London, Oxford University Press, 1949. 101 pp. (Oxford Classical and Philosophical Monographs.) 10s. 6d.

GALEN's concern with Judaism and Christianity was rather superficial. Living in Rome during the latter part of the second century, when public opinion and the imperial administration alike evinced considerable tolerance, even friendliness, toward the numerous faiths in the empire, the influential physician-philosopher could freely associate with members of the Jewish and Christian communities and study their different approach to life with curiosity rather than alarm. The bitterness engendered by the earlier successes of Jewish religious propaganda and the great Jewish uprisings against Rome from 70 to 135 A.D., which had colored the writings of many Roman authors from Cicero to Tacitus, now gave way to a calmer appreciation, if not understanding. Nor did the Roman intelligentsia as yet view the Christian mission as the great menace to the traditional Graeco-Roman way of life which it was to become a century later. Even in Alexandria, long the restless intellectual capital of the eastern Mediterranean, Celsus, author of the first comprehensive defense of Graeco-Roman paganism against the Judeo-Christian outlook on life, had to use many arguments previously assembled by such Jewish apologists as Philo. Another contemporary, Numenius of Apamea, could so sympathetically write about Jews and Judaism that some modern scholars could regard him, however erroneously, as a convert to Judaism.

We may understand, therefore, that living in the western part of the empire where, apart from the imperial capital itself, there were but few and sparse Jewish and Christian communities, Galen mentioned the two faiths only, as Walzer styles it, in a few "asides." But precisely because they were devoid of bitterness, these brief observations often sharply exposed the roots of the basic conflict between scientific evidence and revealed truths. To bridge this gap was to become the major preoccupation of medieval scholastics, and Walzer rightly refers to Maimonides' lengthy answer to Galen's strictures (in *Aphorisms on Medicine*) as an unwitting confirmation of their essential correctness (p. 34).

The author, now serving as lecturer in medieval Arabic and Hebrew Philosophy at the University of Oxford, has culled four interesting passages from Galen's numerous writings, partly extant only in Arabic translations, in which the Roman scientist took direct issue with the Judeo-Christian reliance on Revelation. Galen compared practitioners of medicine without scientific knowledge to Moses "who framed laws for the tribe of the Jews, since it is his method to write without offering proofs, saying, 'God commanded, God spake.'" Like other pagan thinkers, Galen was particularly incensed over the Judeo-Christian doctrines of creation *ex nihilo* and future resurrection, for to Moses "it seems enough to say that God simply willed the arrangement of matter and it was presently arranged in due order for he believes everything to be possible with God. . . . We say that certain things are impossible and that God does not even attempt such things." In another passage, however, Galen admitted that "one

might more easily teach novelties to the followers of Moses and Christ than to the physicians and philosophers who cling fast to their schools." In still another context he spoke admiringly of Christian morality and self-control (Walzer cogently defends the genuineness of this passage).

None of this is startlingly original or brilliant. Nevertheless, Dr. Walzer is to be congratulated on having assembled these quotations from many out-of-the-way places and provided them with an extensive, often searching commentary. His suggestion that Galen's influence made itself immediately felt among some Christian contemporaries, particularly the Adoptionists, and indirectly forced the church, despite its repudiation of the latter, to reconsider its philosophical position, is likewise very fruitful and ought to be further elaborated. The essay concludes with a brief review of "Galen's statements in the Arabic tradition." From all these aspects, the present booklet is an interesting contribution to the history of culture, and particularly of the philosophical and religious debates a century and a half before Constantine, even more than it is to the history of medical science.

New York City SALO W. BARON

B. EBBELL. *Die Alt-Ägyptische Chirurgie*. Skrifter utgitt av Det Norske Videnskaps-Akademi i Oslo. II. Hist.-Filos. Klasse. 1939. No. 2. Oslo, Jacob Dybwad, 1939.

AT present the Norwegian, B. Ebbell, is probably the only scholar who commands with equal competence a professional knowledge of medicine (he is a physician in Oslo) and a thorough understanding of the ancient Egyptian tongue. He has demonstrated this competence in short papers concerned with the interpretation of Egyptian names of diseases, and more recently in a monograph of the Norwegian Academy of Science (Oslo, 1938) on "Altägyptische Bezeichnungen über Krankheiten und Symptome."

The number of ancient Egyptian medical works that have come down to us is very large. These include both those works that can be designated as medical without qualification, as well as those whose content is more magical in character or which belong rather to the area of "folk medicine." Warren R. Dawson has given us an excellent review of this literature in the *Legacy of Egypt,* edited by S. R. K. Glanville. Preëminent in the first group are two substantial papyri, both dating from approximately the same period, the beginning of the 18th Dynasty (about 1500 B.C.). One of these is the *Ebers Papyrus* which has long been published, and has been translated and commented on a number of times. According to reliable reports, the Papyrus which was in the Leipziger Universitätsbibliothek escaped destruction during the war. The second of these is the *Edwin Smith Papyrus* purchased in 1862 in Luxor by the American whose name it bears and brought to the United States. It became a part of the Egyptian col-

lection of the New York Historical Society. Later it was transferred to the Brooklyn Museum and more recently has come into the possession of the Library of the New York Academy of Medicine. Although scientific circles knew about this important medical document, it was not until 1920 that the Papyrus was published by the late James H. Breasted (of the University of Chicago), with a translation, commentary, a vocabulary, and an excellent introduction to orient the reader.

Breasted's achievement in this publication is beyond all praise—but Breasted was an Egyptologist, not a medical man. Although advised by experts, he was not able to interpret the entire medical content of the text. Concerning some descriptions of morbid phenomena he was unable to arrive at a definite opinion; others he misunderstood and, in general, greater or lesser slips in the translation can be demonstrated. It is therefore Ebbell's great merit to have provided a new translation of a large portion of the Papyrus, the important surgical sections, as well as an expert commentary. However, Ebbell did not limit himself to this, but dealt in the same thorough manner with the surgical section of the *Ebers Papyrus* which concerns itself with "swellings" that require surgery. As a result, his treatise is a valuable contribution to the history of surgery. Ebbell points out that the surgical sections of the *Smith Papyrus* cannot be designated as surgical in the strictly scientific sense, but that they might more correctly be described as dealing with the art of wound treatment (*Wundarzneikunst*), since they are concerned with the diagnosis and treatment of wounds of different kinds, fractures, dislocations and similar conditions.

As I am not a physician, it is not for me to check the accuracy of Ebbell's new medical diagnoses and emendations. As an Egyptologist, however, I can say that his translation is completely reliable. Every physician who is interested in the history of his profession, and especially in the knowledge and therapeutic methods of his colleagues more than three thousand years ago, will find Ebbell's book of considerable interest. To such readers it is warmly recommended.

North Hollywood, California GEORGE STEINDORFF

M. D. K. BREMNER. *The Story of Dentistry, from the Dawn of Civilization to the Present.* 2d edition. Brooklyn, N. Y., Dental Items of Interest Publishing Co., 1946. xiii + 332 pp.

BREMNER's *Story of Dentistry* falls far short of fulfilling the claims of its title because it is incomplete, and because many of the statements and dates given in it are inaccurate. The book begins with Adam and Eve, indeed even "Before Adam" if we look at the title of Chapter II (clever but not accurate), and continues not "to the present" as the subtitle has it, but only to the invention of porcelain teeth toward the end of the eighteenth century. Here, after not quite sixty pages of text corresponding to the title, the story breaks off abruptly. The following two

hundred pages comprising more than two-thirds of the book are, however, restricted almost exclusively to a detailed history of dentistry in North America.

Thus, for Bremner and also for his readers every notable achievement in the field of dentistry in Europe ceases with the invention of porcelain teeth in France. Yet anyone who knows the history of dentistry is aware that the practical work of European dentists, and even more so their scientific endeavors, have contributed essentially to the rise and recognition of dentistry. Unquestionably, this belongs in a history of the field which claims to cover the subject to the present.

On the other hand, the first chapter, "From Tubercles to Teeth," in which the development of the teeth but not the development of dentistry is described, definitely does not belong in such a volume. Similarly, the subjects of the fourth and fifth chapters, "Magic in the Treatment of Toothache," and "Old Beliefs about the Teeth," should not be treated in separate chapters torn out of the context necessary for their understanding.

The actual history of dentistry, with the usual descriptions of its practice and its literature, among the various peoples and cultural groups through the centuries is presented at an express tempo in a very compressed form. But the essentials are not emphasized, and often quite unimportant events and men are mentioned. This defect is due to the fact that Bremner has not chosen his sources well and has not understood how to separate the wheat from the chaff. *"The Story of Dentistry,"* Bremner acknowledges in a foreword, "is not a source book but rather a compilation of material from many writers in the literature of dentistry and allied fields." Furthermore, in his bibliography, Bremner cites a series of books and articles which themselves offer no source materials, but are likewise only compilations. On the other hand, the work which would have been most important for his book, the *Geschichte der Zahnheilkunde* by Karl Sudhoff, which is based exclusively on source materials and without which a useful history of dentistry can hardly be written, is not even mentioned. Likewise, the excellent *Documents pour servir à l'histoire de l'art dentaire en France* by Georges Dagen is completely unknown to the author. Thus it is not surprising in view of Bremner's employment of such defective instruments that the result of his effort is quite unsatisfactory. Had Bremner known Sudhoff's as yet unsurpassed work, he would easily have been able to avoid the numerous errors contained in the first part of his story. He would have found that the famous *Artzney Büchlein wider allerlei kranchkeyten und gebrechen der tzeen,* also known as *Zene Artzney,* the first dental monograph in the literature and therefore a landmark in the development of dentistry, first appeared at Leipzig in 1530, and not at the end of the fifteenth century in Basel as he claims. He would also not have been able to maintain that Eustachius (in 1563) wrote "the first book ever published devoted entirely to the dental field." He apparently confuses Guy de Chauliac, who lived in the fourteenth and not the sixteenth century as Bremner claims, with Abulcasis when he wants the reader to believe that Guy de Chauliac "left sketches

of scrapers, elevators, forceps, tooth trephines and files." Guy de Chauliac never left such sketches. On the contrary, they were discovered for the first time in the manuscripts of the *Cyrurgia of Abulcasis*. Among these, however, are not the two tooth brushes, which Bremner depicts in his plate V, erroneously ascribing them to the great Arab physician. According to Bremner, the Roman Cascellius, who lived in the first century A.D., and whom Martial mentions in one of his epigrams, is supposed to be "the first dentist in history whose name has come down to us." However, at another point in his story, Bremner mentions two Egyptian dentists who lived three thousand years earlier, the "toothist" Hesi-Re who was active at the court of the Pharaoh about 3000 B.C., and "chief of the tooth workers," Kuni, who was actually named Khuy.

Such contradictions are to be found in Bremner's work in large numbers, particularly in his dates. These are all the more confusing as he cites different dates for the same events at various points in his book. For example he has the great Pierre Fauchard, the founder of modern dentistry and author of the classical, two-volume work, *Le Chirurgien-Dentiste ou Traité des Dents* (not, as he has it, *Chirurgien Dentiste au traite des Dentes*) die twice on two successive pages of his book, once in 1758, the second time, three years later, in 1761.

The number of such errors in Bremner's story is so extensive that it is not possible to list them in a review without boring the reader, who has no very great interest in such corrections. But Bremner's speculations and conclusions often give a completely false picture of actual events and conditions. When, in connection with the disturbed periods of the medieval crusades, he says, "those turbulent centuries were hardly conducive to the development of the pursuit of peace", and follows it by the assertion that "dentistry could thrive only in a climate of prosperity and tranquillity", then one must ask how Bremner conceives of these relations. If he means the progress of scientific dentistry, he must be reminded that science has developed independently of prosperity and tranquillity, and that the greatest discoveries and inventions have been made even in times of unrest and poverty, both general and personal. And if he means progress in the field of technical dentistry, he should remember that the enormous advance of dentistry during the last century is due almost exclusively to the development of general technology, and has nothing to do with prosperity and tranquillity. All dental technical innovations have been borrowed from general technology and adapted to the special requirements of dentistry. It was the recognition that rubber could be vulcanized and used for dental plates which first made possible "False Teeth for the Millions," as Bremner strikingly expresses it in the title of one chapter. Similarly, it was the production of precision instruments which made it possible for the dentist to do exact work in the patient's mouth and in the dental laboratory, something which until then was not possible with the dentist's inadequate instrumentarium and imperfect apparatus.

In the first part of his book, Bremner has ventured into an area which

requires more specialized knowledge, particularly of the sources, than he exhibits. Furthermore, people in glass houses should not throw stones: "Having nothing original to say has not necessarily inhibited some dentists from writing" (Bremner, p. 283).

While the first part of this *Story of Dentistry* is unsatisfactory, the depiction of the development of dentistry in North America is instructive and interesting. Had the author restricted his book to "The Story of North American Dentistry," and either omitted the first part completely or, without the errors in it, used it as a short introduction to a regional history of dentistry, there would have been every reason to welcome his work. Bremner has a very witty and humorous style, and this not only makes the subject attractive and interesting for the "average man in practice or the student" for whom the book is intended, but also for those better acquainted with the subject. It is truly a pleasure to read his dramatic and exciting account of the development of the gold inlay technique, and the subsequent patent fight of the Chicago dentist, Dr. William H. Taggart; or the story of the discovery of anesthesia with all its dramatic and tragic circumstances. The founding of the first dental journals, dental schools, and dental organizations as well as the necessary consideration of the "business side of dentistry" are treated in detail. Finally, there are two chapters on women in dentistry, and on G. V. Black, the many-sided "Grand Old Man of Dentistry," one of the most interesting personalities of the dental profession during the last quarter of the nineteenth and the first decade and a half of the twentieth centuries.

The chronology placed at the end of the book is not always accurate as far as the period up to the beginning of the nineteenth century is concerned, quite apart from the fact that it is not arranged according to well-established dates, but rather according to entire centuries.

New York City CURT PROSKAUER

BENJAMIN FARRINGTON. *Francis Bacon, Philosopher of Industrial Science*. New York, Henry Schuman, 1949. xiii + 202 pp. Illus. $3.50.

IT is a significant achievement to array fresh data and interpretations when writing about Francis Bacon who has been so widely discussed for three centuries. Yet Professor Farrington, who is professor of classics at University College, Swansea, Wales has succeeded in doing this in his discerning, cogent biography. With rare clarity of exposition, and often brilliant prose, Farrington has given a sympathetic yet critical appraisal of Bacon's intellectual stature in the perspectives of his period. His book is not a conventional reiteration of the praise or censure of earlier authorities, but a revaluation of the work of Bacon into a new and discerning pattern of analysis.

Bacon is worthy of such an analysis in spite of his limitations. His break with speculative philosophy marked an important event in intellectual history,

with the issues in the controversy still largely unresolved. Farrington is particularly rich in his interpretation of the meaning of this controversy. He sees Bacon as the pioneer thinker in a period of revolutionary change in production, who was convinced men must consult nature rather than books (to make books out of life, rather than books out of books) if they are to make progress in truth which to him was the power to improve the conditions of human life. Farrington aptly characterizes Bacon as "philosopher of industrial science," and declares, "The organizing principle of all his vast erudition was the aim of extending, not man's power of argument, but his power of action. But the special character of his enterprise [the new encyclopædia, *The Great Instauration*] was his insistence that a reform in practice depended on a reform in thought. So sure was he of this that he constantly identified the two processes, insisting that what is successful in practice is true in theory, that progress in power and progress in knowledge are two aspects of the one thing. It is a mistake to confound this with utilitarianism. For Bacon, works were the test of truth; he did not say they had value above truth."

It is in this frame of reference that Farrington finds many of Bacon's interpreters wanting. It has been customary for scholars to concentrate on the logical aspect of Bacon's work with special emphasis on *Novum Organum,* and to discuss him as "a devisor of rules of thinking" coming between Aristotle and John Stuart Mill. Farrington stresses the fact that *Novum Organum* was never published alone but formed a deliberately abandoned fragment of his encyclopædia, *The Great Instauration.* The aim of this novel work designed to contain experimental history, which to Bacon meant a record of all man's successful efforts to exercise practical control over nature, was not to teach men how to argue correctly but how to invent new arts. Bacon's objective was not only to reform logic but also to transform the material conditions of life in the interests of man. Farrington indicates that the measure of truth in Harvey's oft-repeated gibe that Bacon wrote philosophy like a Lord Chancellor lies in the fact that Bacon's works are formulated by a person who thought in terms of projects for execution rather than conceiving merely of books to be read. He thus sees Bacon's claim to fame as a logician dubious, but considers him to be the herald of the industrial revolution "who foresaw the possibility and consequences of the application of science to industry and pleaded with his countrymen and with the world to stretch their moral and intellectual capacities to meet the new challenge."

Bacon's vigorous attack upon Aristotle and Plato is brought into better focus in this setting. Farrington shows that Bacon considered them men with outstanding mental gifts whose systems of thought served as the chief impediments to the foundation of a true philosophy of nature, that he was a pioneer in noting that whereas philosophy is stationary, applied science is progressive. Farrington puts it this way: "The raw material for the contemplative science of Aristotle could be gathered by the contemplation of nature. The raw material

for the active philosophy of Bacon could be gathered only from the examination of man's action on nature. . . . Aristotle's logic was an aid to thinking; its goal was logical consistency. Bacon's logic was a guide to action; its test was whether it worked." The ancient philosophers were to Bacon intellectually brilliant but morally wrong because they had achieved little to improve man's lot through a better understanding of nature. He proposed instead to take a vast inventory of natural phenomena by means of the organized teamwork of many men to the end that man would not only understand nature but control it. Farrington sketches the institutional setting for this intellectual conflict. The philosophy of the supporters of feudalism which stood in the way of the new bourgeois England was the Aristotelianism of the Schools. The struggle for science in industry therefore became associated in Bacon's mind with the reform of philosophy.

Farrington notes the unresolved contradictions in Bacon's outlook that checked the full development of this thought. Among the most important of these is his absolute division between natural and divine knowledge which lead him to give directions for the advancement of natural knowledge but to teach that divine truth should be accepted uncritically as literally revealed in the Bible. Another is that conveyed by the title of *The Great Instauration,* that there once existed a higher state of human wisdom which needed to be restored, a view which ran contrary to another of his doctrines that modern times are wiser than the old. The author also gives evidence that Bacon underestimated the work to be done and overestimated his own powers.

In his criticism, as in his praise, of Bacon, Farrington manifests an exemplary level of judicious scholarship which makes his contribution to the Life of Science Library a noteworthy event.

New York City BERNHARD J. STERN

WILLIAM DOOLIN. *Wayfarers in Medicine.* London, William Heinemann Medical Books Ltd., 1947. 284 pp. Illus. 21*s.*

Wayfarers in Medicine is a delightful collection of essays which were originally published in *The Irish Journal of Medical Science* and are here reissued in a handsome volume. The author, Mr. William Doolin, is a Dublin surgeon. For near to a quarter of a century he served as the editor of the journal in which the essays were originally published.

This collection of essays makes pleasant reading. The author has a facile pen; he is urbane, well read, and genial. In the main he has retold the tales in medical history that have been told many times before. But he has done so with skill and wit and in good prose. Even when the material is well known, it is yet a pleasant experience to see the recitation unfold. But the appeal is chiefly to the eye and ear. The author is not inclined to dig below the surface. And when

he does allow himself some generalizations, or offers some observation "aside," he is not at his best, nor is he always convincing. Indeed his facile and sweeping observations are at times quite irritating. Thus he characterizes Asclepiades of Bithynia as a shrewd and able practitioner who owed his reputation and success to the liberal use of wine, diet, massage, and baths. This of the "Prince of Physicians"! He assures us, without citing his authority, that "dissection of the human body was expressly forbidden to the ancient Greeks by their religion." (p. 161) The decline of surgery in medieval times he ascribes to a misinterpretation of Galen's dictum that surgery was only a method of treatment, which misunderstanding led the "schoolmen to thrust out the surgeon from their midst—to their own detriment."

Perhaps it is precisely because the author is a surgeon and an adherer to the Church of Rome that he is so far off in his recitations on the history of surgery. In his version the decline of surgery is attributable in the main to the influence of the Arabs. "Arabic physicians were the first to inculcate the doctrine of the inferiority of the surgical branch of the healing art, which unhappily persisted for so many centuries after the expulsion of the Moslem from Western Europe"; "Arabic influence must be held primarily responsible for the lack of surgical development between the 7th and 12th centuries." (p. 165) The contribution of the Church to the decline of surgery by its prohibition of the practice of surgery by the clergy the author considers to have been nugatory and mostly misunderstood and misinterpreted.

But these lapses and biased versions of some few elements in the history of medicine do not substantially detract from the pleasure that the rest and better parts of this book afford. Doolin is at his best when he has culled some medical oddments while reading in general literature. The *Vita* of Cellini serves him as the matrix for a fine essay on "A Patient of the Renaissance." Equally delightful are the essays, "The Anatomist in Art," "Some Antique Stones," and "Some Old Journeymen Surgeons." Doolin disclaims in great modesty any expertness in art matters, yet his essays reveal a fine knowledge and a keen appreciation of the graphic arts, at least in so far as these have a relation to matters medico-historical.

All in all the book contains twenty-seven essays, and it is fitting that the twenty-seventh should deal with "Pathfinders in Dublin." Save for the several defects already noted, *Wayfarers in Medicine* is a book that evokes a kindly and warm appreciation. It were a rudeness not to make note of the author's protest, "My trifling with the history of medicine has been . . . an exercise devoid of any pretence to scholarship or research: these essays were prepared simply for the evening's entertainment. . . ." That they surely afford, and more.

New York City IAGO GALDSTON

ELIZABETH H. THOMSON. *Harvey Cushing, Surgeon, Author, Artist.* New York, Henry Schuman, 1950. xviii + 347 pp., 12 pl., 13 illus. $4.00.

BORN in Cleveland, Ohio, on April 8, 1869, of a line of distinguished physicians, Harvey Cushing became one of the truly great of the world's surgeons. It was his special goal in life to establish a school of surgery of the nervous system; to make surgery of the brain as safe as that of other parts of the body. Previously, this had been a part of the field of general surgery, its basic problems beyond the patience and the abilities of the majority of its practitioners. The task required the genius of such a man as Harvey Cushing.

His handling of the task was so preëminent that he was honored by his profession, not only in his own country, but in the world at large. His laurels he wore lightly and with distinction. During his active years he graced the faculties of Johns Hopkins and Harvard and finally of Yale, his beloved Alma Mater.

In addition to his professional abilities, Cushing was an artist of no mean proportions and an author whose work merited the Pulitzer Prize in Letters. He was a bibliophile whose infectious energies with the co-operation of Drs. John F. Fulton and Arnold C. Klebs of Nyon resulted in the establishment of the Historical Library of the Yale University School of Medicine. He died shortly after arranging this, on October 7, 1939.

The complexities of his personality as well as the sheer bulk of material to be handled provide his biographers with an awesome task as well as a sparkling challenge. His first biography published in 1946 was written by Dr. Fulton, who had been a long-time though younger friend, companion, and fellow bibliophile. This is a large work and, while delightfully told, might, by its size, "put off" the general reader and the young physician and surgeon.

Miss Elizabeth Thomson helped Dr. Fulton prepare his biography for the press and as a research assistant at the Historical Library was especially qualified to undertake the writing of a shorter story of Dr. Cushing's life. She had the added advantage of a viewpoint more objective than that of one who has been so near the subject of his study. She also was able to portray more clearly the great influence of Mrs. Cushing upon her husband's personal life and his career. During her lifetime, Dr. Fulton had not been able to emphasize adequately Mrs. Cushing's rôle. Miss Thomson has used fresh material in her book, especially that concerning Dr. Fulton. She has told the story skillfully and well. This biography makes a notable addition to the Life of Science Library for which it was written and should have the wide attention that it merits.

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